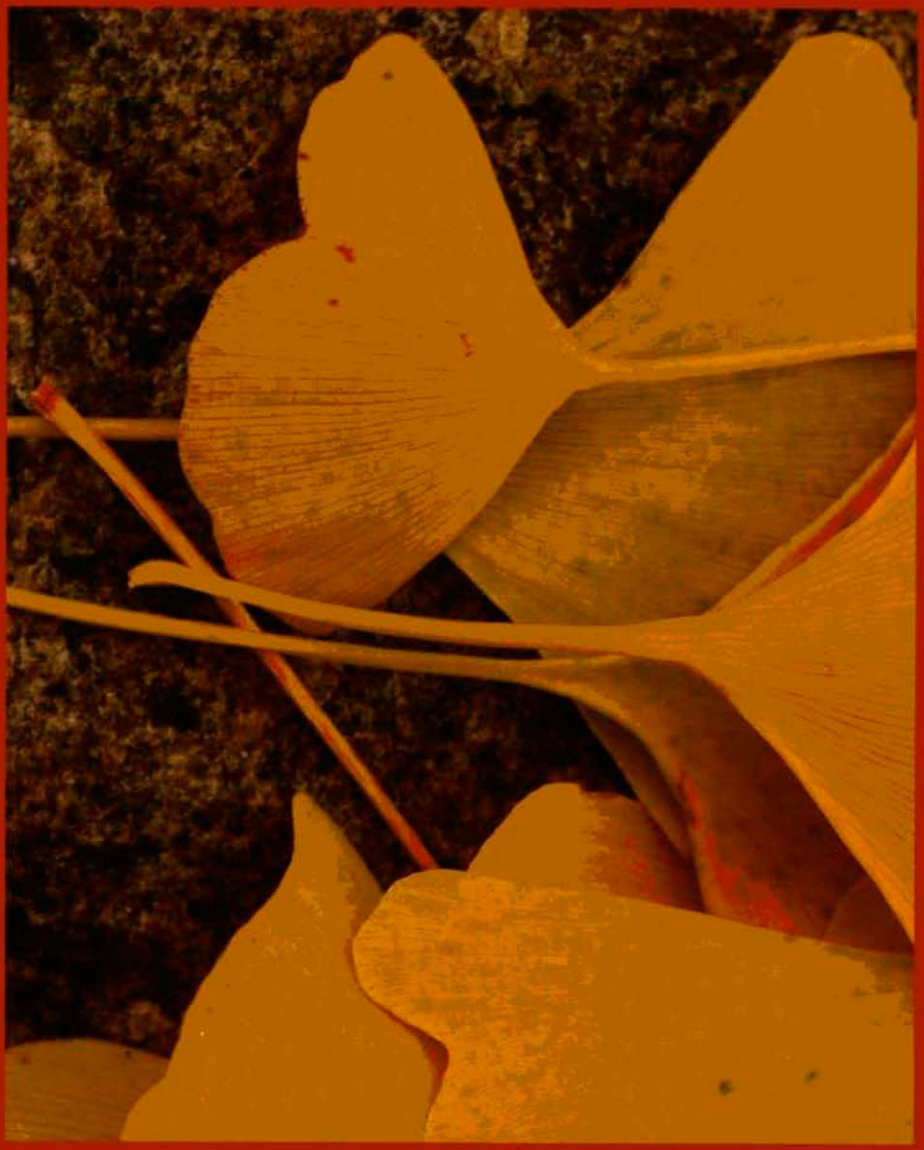
Acta Universitatis Szegediensis

Acta Biologica Szegediensis

Volume 53, Suppl. 1, 2009



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Acta Biologica Szegediensis

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5th Congress of the Hungarian Society for Free Radical Research

27-29 August 2009
Szeged, Hungary

On the cover: *Ginkgo biloba*, one of the oldest living plant species, has natural antioxidant effect.

PREFACE

The present volume, a Supplement of Acta Biologica Szegediensis involves the proceedings for the 5th Congress of the Hungarian Society for Free Radical Research, held in Szeged, Hungary, 27-29 August, 2009.

It contains contributions from several special fields of free radical research, namely, molecular base of oxidative and nitrosative stress; role of free radicals in the patomechanism of diseases; oxidative damages and antioxidant defence systems is plants, animals and human; oxidative stress, life style and natural antioxidants; neurodegeneration, and oxidative processes; new methods for determination of free radical damages.

The papers contained by the current volume, although represent only a part of the oral and poster presentations, offer a good overview of this unique conference. There were participant from several field of sciences; physicians, biologists, molecular biologists, veterinary, agrarians, chemists. The connection between them is the common research topic, but different aspects.

The history of free radical research in Hungary is very rich and eventful.

The birth date of free radical research in Hungary is 1978 – almost three decade before. That time was organised the first round table conference in Szeged according to proposition Prof. Béla Matkovics. Interest was growing up from time to time and more and more scientist joined to free radical research. Next two-days conference was held in Pécs in the year 1984 organised by Prof. Béla Török. Two years later in 1986 Prof. Matkovics Béla organised conference in Szeged entitled "Oxygen free radicals and tissue damage". In 1989 two big Hungarian towns – Debrecen and Szeged – organised a conference together. Proceedings of the conference was published by the Akadémiai Kiadó (Budapest) entitled "Radicals, ions and tissue damage". In 1991 Prof. János Fehér and Dr. Anna Blázovics organised conference in Balatonaliga entitled "Role of free radicals in biological systems". The Free Radical Section of the Liver Foundation was established in that year, too. Our Free Radical Society was joined to the Society for Free Radical Research European Region (SFRR Europe) in 1992 in Turin. From this year our conferences were organised with international participants. For example the conference entitled "Oxygen free radicals and scavengers in the biological and medical sciences" was organised by Prof. Gyula Mózsik and Dr. Áron Vincze in the year 1993. Important date of our society was the year 1995, when the President of SFRR Europe – Prof. G. Poli asked the Hungarian Section to organise a Summer School in Budapest. Organisers were Prof. János Fehér and Dr. Anna Blázovics. Next symposium was held together with 26th membrane Transport Conference in Sümeg in 1996 organised by Prof. Sándor Imre from Debrecen University of Medicine. At the end of August, 1997 Prof. Miklós Mézes organised the 5th Free Radical Conference in Gödöllő at the University of Agricultural Sciences.

Next years gave us a few less possibilities to hold conferences, but we met almost every year. In the even years one day workshops (mostly in Budapest organised by Dr. Anna Blázovics), every two years two and half days conferences were set up in the different towns (Szeged – 2002, Debrecen-2005, Pécs – 2007) .

There is an interesting situation this year, because our conference is 5th again. The cause is that the society is renewed as Hungarian Society of Free Radical Research at the end of nineties.

We have established the "Matkovics Béla" Plaquette for those who made their best in the working of Hungarian Society of Free Radical Research. First researcher who got this plaquette in 2007 was Prof. János Fehér, everlasting honorary president of our society. This year in "Szeged Conference" prize-winner was Prof. Anna Blázovics, present vice-president.

I express my gratitude and thanks to my colleagues, people and institutions who helped to organize this Congress.

Dr. Ilona Szőllősi Varga
organizer

Volume 53(Suppl.1): 2009
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ARTICLE

Redox homeostasis in gastrointestinal diseases

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ABSTRACT A lot of information is spread all over the world by papers and by other media about oxidative stress and antioxidant defence as well as their connection to human health and diseases, although only a few examine redox homeostasis from this point of view, because of expenses. We offer a cost efficient simple methodological triad "DPPH radical scavenging ability, reducing power and induced chemiluminescent intensity" in plasma and red blood cells as a program to evaluate the individual requirements for correct self control. We are able to evaluate with these global methods the differences between redox homeostasis of inactive, moderate and severe phase of IBD patients, a circadian rhythm in seasons of patients, deviant food consumes and initial state of tumourous processes as well as post operative and metastatic states.

Acta Biol Szeged 53(Suppl.1): (2009)

KEY WORDS

DPPH radical scavenging ability
reducing power
induced chemiluminescent intensity
gastrointestinal diseases

Redox homeostasis can be considered as the cumulative action of free radicals and antioxidant defences, providing a suitable condition for life (Blázovics 2007). Moderate oxidative stress is important in signal transduction pathways and essential for proliferation and apoptosis. Oxidative stress can induce stress response genes, and moderate oxidative stress by down regulating the gene expression of several genes. DNA synthesis, selective gene expression, enzyme activation and modification of cell proliferation are involved in redoxi signal mechanisms. Moderate free radical production can modify the function of kinases or directly activate the transcription factors, thereby also influencing the gene regulation in the nucleus (Powis et al. 1997; Suzuki et al. 1997; Sebolt-Leopold et al. 1999; Straus et al. 2000; Haddad 2002; Horbinski and Chu 2005).

The presence and absence of some transition metal and non-metal elements significantly modify the signal transduction processes therefore, their optimal tissue concentrations are not doubtful (Pena et al. 1999; Kudrin 2000).

Redox homeostasis can be examined with a cost efficient simple methodological triad "DPPH radical scavenging ability, reducing power and induced chemiluminescent intensity" in plasma and red blood cells, to evaluate the individual requirements for correct self control. Applied with these methods differences of inactive, moderate and severe phases during applied therapy in IBD patients vs. healthy controls could be made. Erythrocyte scavenging function is significantly lower in the severe and moderate phases of Crohn's disease and slightly lower in the inactive stage. Similar to the control, the patients with inactive ulcerative colitis have a better redox status of red blood cells

(Blázovics et al. 1999). A circadian rhythm in the measured parameters in seasons of patients could be found. During summer months both the defence mechanism and the free radical activity differ from those of winter months (Blázovics et al. 2007). Deviant food consumers among patients also could be picked (Blázovics et al. 2004). The results were correlated with laboratory parameters and element concentrations of Caucasian IBD patients and healthy volunteers in both genders. The antioxidant defence system is partly related to element status via enzyme activity and uncontrolled free radical reactions (Pena et al. 1999; Szentmihályi et al. 2008).

The aim of this study was to examine the differences of redox homeostasis of healthy control, other patient control, IBD patients, discovered tumourous patients, treated tumourous patients and patients suffered from metastasis with these global methods and to make a correlation with other laboratory parameters, as well as to make an expense-reducing, methodical offer to survey their conditions.

Materials and Methods

1,1-diphenyl-2-picrylhydrasyl stable radical, luminol, hydrogen peroxide and microperoxidase were obtained from SIGMA (St. Louis). Tumour markers, CEA, CA 19-9, AFP kits (LIA-mAT immunoluminometry) were obtained from LIA-mAT (Budapest). CRP (CRP/AUT-000) was obtained from Diagnosticum Ltd. All other reagents in analytical stage were purchased from Reanal (Budapest).

Patients: Adult Caucasian (years: 25 - 60) volunteers from both genders, healthy controls (N = 10), patient controls (N =

Accepted June 19, 2009
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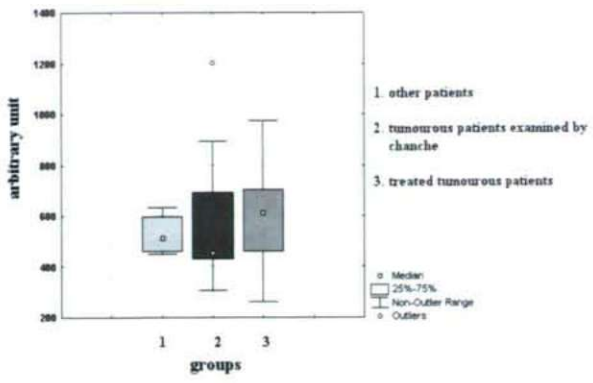

Figure 1. Glutathione peroxidase activity of red blood cells.

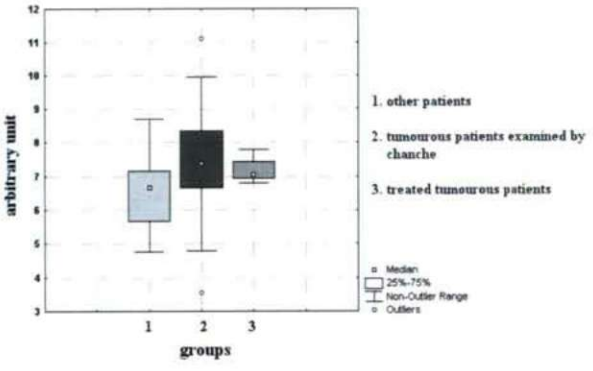

Figure 2. Superoxide dismutase activity of red blood cells.

22), discovered tumourous patients (N = 8), treated tumourous patients (N = 27), patients with metastasis (N = 6) and IBD patients (N = 113) including ulcerative colitis (34), Crohn's disease (79) were drawn into the study. They underwent routine examinations as well as 3D abdominal ultrasound examinations. Patients with positive routine or 3D ultrasound examinations underwent further examinations (endoscopy and

Table 1. Significant correlations in tumourous patients.

variable	Correlations of redox parameters of treated tumourous patients. Marked correlations are significant at p<0.05					
	RBCCL	TAS	Bile acid	SOD	GSHPx	Plas-maCL
RBCCL	1.00	0.14	-0.22	-0.26	-0.06	0.14
TAS	0.14	1.00	0.15	-0.13	0.09	-0.19
Bile acid	-0.22	0.15	1.00	-0.27	-0.24	-0.47
SOD	-0.26	-0,13	-0.27	1.00	0.47	0.38
GSHPx	-0.06	0.09	-0.24	0.47	1.00	0.29
PlasmaCL	0.14	-0.19	-0.47	0.38	0.19	1.00

RBCCL = red blood cell chemiluminescence; TAS = total antioxidant status; PlasmaCL = plasma chemiluminescence.

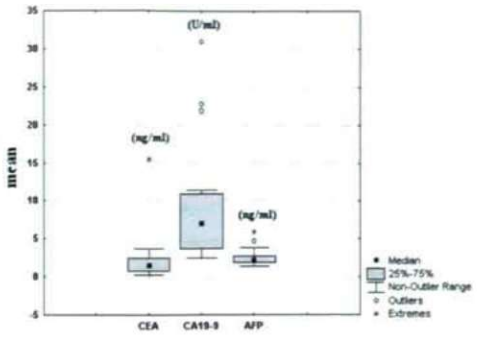

Figure 3. Tumour markers in other patients without tumours.

CT). Patients with Dukes B and C received the same recommended standard therapy after operation.

Permission number: TUKEB 167/1997; 15/2004 and IKEB 3944/2004.

Sera, plasma and red blood cells were separated using standard methods with centrifuge at 2500 rpm at 4°C. The haemoglobin content was adjusted to 10 g% uniformly for the measurements.

Routine laboratory parameters of sera were measured by Roche enzymatic in vitro assays. Tumour markers (CEA, CA 19-9, AFP / Berthold Lumat 9501 manual instrument), CRP, redox parameters were measured with routine laboratory parameters together. Plasma TAS: Randox® kit (Cat No. NX2332), superoxide dismutase RANSOD (SD125) and glutathione peroxidase RANSEL (RS505) were applied. (Randox laboratories, Ltd, Crumlin, UK).

Plasma hydrogen-donating ability (PHDA) was estimated in the presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of Hatano et al. (1988).

Oyaizu's method was adopted for the analysis of the reducing power of the plasma (PRP). The change in absorbance was measured, which accompanied Fe^{3+}-Fe^{2+} transformation at 700 nm, and the (PRP) was compared to that of ascorbic acid (Oyaizu 1986).

The chemiluminescence assay adapted to a Berthold Lumat 9501 instrument, which was applied for the determination of the total scavenger capacity of the plasma and red blood cells, to assess the antioxidant deficiency in patients with intestinal diseases. The scavenger capacity of the samples obtained from healthy individuals and patients were expressed in RLU (relative light unit) of the standard (basic chemical reaction; Blázovics et al. 1999).

Statistical analysis: All clinical tests were expressed as mean and standard deviation (SD). One-way ANOVA statistical analysis was applied to evaluate the significance between patient groups. Each measuring point represents five parallel data in luminol-dependent chemiluminescence experiments when c.v.% was under 5.00%. A value of $P<0.05$ was accepted as statistically significant.

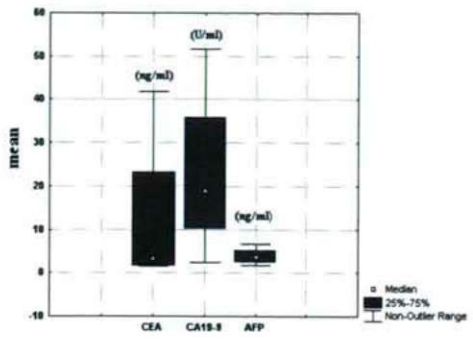

Figure 4. Tumour markers in colon tumourous patients examined by chance.

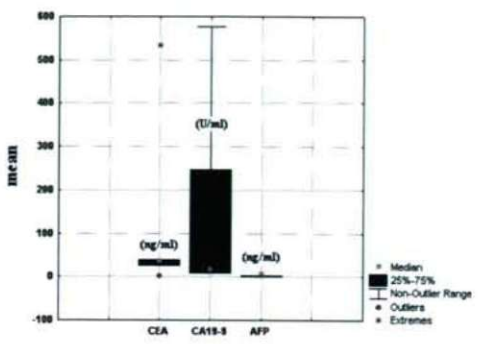

Figure 6. Tumour markers in colon metastases.

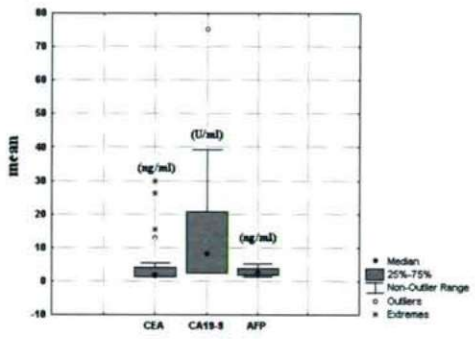

Figure 5. Tumour markers in treated colon tumourous patients.

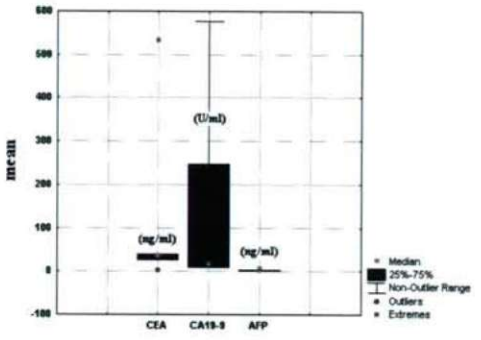

Figure 7. Stimulated chemiluminescence in red blood cells of patients with different gastrointestinal diseases.

Results and Discussion

Plasma-TAS, reducing power, H-donating ability and red blood cell GSHPx, SOD and chemiluminescence intensity (RLU) as well as serum tumour markers and laboratory parameters were determined.

Neither the activity of SOD nor GSHPx of red blood cells showed significant difference in different stages of tumourous patients, although variance was large (Figures 1. and 2.).

The results of tumour marker examinations strengthened that tumour markers showed large difference in different stages, although the highest in metastasis. Therefore tumour marker examinations are doubtful (Figures 3-6.).

We could not find any correlation between TAS or red blood cell RLU vs. tumour markers as well as between other redox parameters in cancer, whereas the correlations between redox parameters of female Crohn's patients were significant:

Table 2. Significant correlations in IBD.

positive correlation		negative correlation	
UA–HGB	($r = 0.7913$; $p = 0.004$)	RBCCL–UA	($r = -0.7743$; $p = 0.009$)
UA–HCT	($r = 0.8332$; $p = 0.001$)	RBCCL – HGB	($r = -0.8214$; $p = 0.004$)
UA–PHDA	($r = 0.6328$; $p = 0.020$)	RBCCL – CRP	($r = -0.8987$; $p < 10^{-4}$)
UA–PRP	($r = 0.6229$; $p < 10^{-4}$)	RBCCL – PHDA	($r = -0.8281$; $p = 0.003$)
PFSHG–Na	($r = 0.7472$; $p = 0.013$)	RBCCL –PRP	($r = -0.8942$; $p < 10^{-4}$)
PHDA– PRP	($r = 0.8780$; $p < 10^{-4}$)	RBCCL –HTC	($r = -0.8131$; $p = 0.004$)
RBC–HCT	($r = 0.6103$; $p = 0.046$)	PHDA – TP	($r = -0.5783$; $p = 0.038$)
PHDA-K	($r = 0.7040$; $p = 0.016$)		

CRP = C-reactive protein; RBCCL = red blood cell chemiluminescence; HGB = haemoglobin; HTC = haematocrit; K = potassium; Na = sodium; PFSHG = plasma free SH group; PHDA = plazma H-donating ability; PRP = plasma reducing power; RBC = red blood cell; TP = total protein; UA = uric acid.

red blood cell SOD vs. GSHPx activities (r = 0,76), plasma RLU vs. H-donating ability (r = -0,73). In male Crohn's patients the correlation was weak: red blood cell SOD vs. plasma RLU (r = -0,51).

The correlations between parameters of plasma and red blood cells were the most weak in cancer cases. In the case of tumours in both genders, the results made us conclude that tumour growth and spreading damage components of the defence system seriously (Table 1.) and see Figure 7.

Reducing power and H-donating ability were significantly low in tumorous processes, and the reducing power was not changed significantly in different stage of IBD vs. healthy controls (174,54 ± 22,14 nmol AS). H-donating ability changed in control (60,62 ± 1,98%) vs. inactive (45,88 ± 3,0%) vs. severe (45,44 ± 3,00%) ulcerative colitis and in control (60,62 ± 1,98%) vs. severe (42,86 ± 4,52%) in Crohn's disease.

Plasma RLU vs. bile acid concentrations (r = -0,47) and red blood cell SOD vs. GSHPx (r = 0,47) activities ($p<0,050$) showed only weak correlations in cancerous patients.

The significant correlations in IBD can be seen in Table 2.

On the basis of results of red blood cell chemiluminescence examinations, significant difference could be established between patients with IBD and patients with colon cancer (Figure 7.). We could pick freshly discovered tumours and serious metastases.

Our previous results showed that in tumorous patients the protoporfirin IX – according to concentration – induces free radicals in small concentration and scavenges in higher concentration. At the same time, beside the high protoporphyrin concentration, HCHO (mobilized methyl group) concentration was significantly low in metastatic tumorous patients (Blázovics et al. 2008). (It was verified by Stryeer (1988), that arginine (38), methionine (65) and lysine (72) near the methionine (80) are methylated and coordinated towards the central iron of heme). Therefore it can be established, that in an early state of tumourous processes a low concentration of free protoporphyrin causes an extreme high free radical level, and the high concentration of protoporphyrin in metastasis causes a high antioxidant activity in our experimental system.

The examination of redox homeostasis will bring us closer to know more about tumorous inclinations.

Acknowledgements

Authors express their thanks to Mrs. Sarolta Bárkovits, Edina Pintér for their excellent technical assistances. The research was supported by ETT 12/2006 projects.

References

Blázovics A (2007) Redox homeostasis, bioactive agents and transduction therapy. Curr Signal Transduct Ther Bentham Sci Publisher 2:226-239.

Blázovics A, Kovács Á, Lugasi A (2007) The effect of short and long term antioxidant treatments on redox homeostasis in experimental and clinical studies. In: Nutritional Research Advances, Editor: Sarah Watkins, Nova Science Publisher ISBN 978-1-60021-516-2, Chapter 4., pp. 1-34.

Blázovics A, Kovács Á, Lugasi A, Hagymási K, Bíró L, Fehér J (1999) Antioxidant defense in erythrocytes and plasma of patients with active and quiescent Crohn's disease and ulcerative colitis: A chemiluminescence study. Clin Chem 45:895-896.

Blázovics A, Szentmihályi K, Vinkler P, Kovács Á (2004) Zn overdose may cause disturbance in the iron metabolism. Trace Elem Electrol 21:240-247.

Blázovics A, Szilvás Á, Székely Gy, Tordai E, Székely E, Czabai G, Pallai Zs, Sárdi É (2008) Important bioactive molecules of erythrocytes in colorectal cancer patients after colectomy. The Open Med Chem J 2:6-10.

Haddad JJ (2002) Oxygen-sensitive pro-iflammatory cytokines, apoptosis signaling and redox-responsive transcription factors in development and pathophysiology. Cytokines Cell Mol Ther 7:1-14.

Hatano T, Kagawa H, Yasuhara T, Okuda T (1988) Two new flavonoids and other constituents in licore root: their relative astringency and radical scavenging effects. Chem Pharm Bull 36:2090-2097.

Horbinski C, Chu CT (2005) Kinase signaling cascades in the mitochondrion: a matter of life or death. Free Rad Biol Med 38:2-11.

Kudrin AV (2000) Trace elements in regulation of NF-κB activity. J Trace Elem Med Biol 14:129-142.

Oyaizu M (1986) Studies on products of browning reaction prepared from glucosamine. Jpn J Nutr 44:307-315.

Pena MM, Lee J, Thiele DJ (1999) A delicate balance: homeostatic control of copper uptake and distribution. J Nutr 129:1251-60.

Powis G, Gasdanska JR, Baker A (1997) Redox signalling and the control of cell growth and death. Adv Pharmacol 38:329-358.

Sebolt-Leopold JS, Dudley DT, Herrera R, Van Becelaere K, Wiland A, Gowan RC, Tecle H, Barrett SD, Bridges A, Przybranowski S, Leopold WR, Saltiel AR (1999) Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. Nat Med 5:810-816.

Stryeer L (1988) Biochemistry, 3rd ed.; Freeman and Co., New York.

Straus DS, Pascual G, Li M, Welch JS, Ricote M, Hsiang CH, Sengchanthalangsy LL, Ghosh G, Glass CK (2000) 15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappa B signaling pathway. Proc Natl Acad Sci USA 25:4844-4849.

Suzuki YJ, Forman HJ, Sevanian A (1997) Oxidants as stimulators of signal transduction. Free Rad Biol Med 22:269-285.

Szentmihályi K, Kovács Á, Rapavi E, Váli L, Molnár J, Blázovics A (2008) Element concentration in erythrocyte in moderately active ulcerative colitis by the supplementary treatment with remedy containing black radish root. Trace Elem Electrol 25:69-74.

Volume 53(Suppl.1): 2009
Acta Biologica Szegediensis
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ARTICLE

Negative element balance according to a survey for consumption of some essential elements in cases of patients with inflammatory bowel diseases

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ABSTRACT Malnutrition is a characteristic feature of inflammatory bowel diseases (IBD), often due to unhealthy nutritional habits. Therefore, nutritional habits (intake of vegetables and fruits) and element content in erythrocytes have been investigated. 50 IBD patients (25 male, 25 female) and 50 healthy volunteers (35 male, 15 female) were asked to complete a questionnaire. In addition to routine laboratory parameters, Ca, Cu, Fe, Mg, Mn, P, S and Zn content in erythrocytes were determined with ICP-OES. Decreased level of Ca (0.975 ± 0.440 μg/g), Mg (1.02 ± 0.24 μg/g) and Zn (0.776 ± 0.482 μg/g) was observed in IBD patients at $P<0.05$ level compared to the control (2.90 ± 2.25 μg/g, 18.28 ± 9.66 μg/g and 1.05 ± 0.48 μg/g). IBD patients consume similar foodstuffs to healthy people although in lesser amount. The intake of nutritional antioxidants was almost the same in both groups, whereas element intake differed because of diverse nutritional habits. According to the survey, in Hungary healthy people consume about 18-66% of essential element requirements. In the case of IBD patients the situation is worse (15-60%) because of lesser intake and malabsorption. Lowest element intakes were observed for Ca and Zn. The mineral element imbalance in IBD patients probably contributes to their deficiency. Since IBD patients and the controls are on similar diet, latent element deficiency may develop in healthy volunteers which may enhance the risk of metabolic diseases.
Acta Biol Szeged 53(Suppl.1): (2009)

KEY WORDS

nutritional habit
malnutrition
IBD
Ca-
Mg-
Zn-
Fe

Malnutrition is a serious problem in inflammatory bowel diseases (IBD) e.g. ulcerative colitis and Crohn's disease. Since intestinal permeability is altered, the absorption and bioavailability of different nutritional components e.g vitamins and metal elements (Fe, Zn, Ca, Mg, K) are also changed (Goldschmid and Graham 1989; Bhaskar et al. 1995; Blázovics et al. 2000; Bruwer et al. 2001; et al.; Gasche et al. 2004; Blázovics et al. 2006). As a result, multiple deficiency state may develop and IBD patients may need adequate substitution of different essential agents (Sturniolo et al. 1998; Blázovics et al. 1999a). In deficiency state, the therapy is completed with mineral supplementation but in spite of this, metal element homeostasis often remains modified. In some cases significant lower Fe and Se concentration was measured in the plasma of IBD patients. In other cases, however, no significant difference was found in the Se content (Sturniolo et al. 1998). Since the element balance of IBD patients is also dependent on the severity of the disease, comparison of the data is very difficult.

Accepted June 19, 2009
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As is known, redox homeostasis is altered in IBD patients (Blázovics et al. 1999a, 2004a). Metal elements play an essential role in the human body and in redox homeostasis. The quality and quantity of participating elements alter the redox homeostasis of tissue, cell and subcellular particle of specifically. Both oxidative and antioxidative processes may catalyzed by some elements (Cu, Fe, Mn, Zn). The role of P, S and Se is also very important in the antioxidant function. NF-κB, JNK and p53 signaling proteins play an important role in apoptotic death and free radicals and metals are significant mediators in the apoptotic process as well (Kudrin 2000; Sheikh and Fornace 2000). Transition metal ions are ubiquitous in biological systems. The presence and absence of some transition metal (Cu, Fe, Mn, Zn) and non-metal elements (P, S, Se) significantly modify the signal transduction processes, therefore, optimal tissue concentration is, no doubt, highly important. The most favorable results were attained by substitution of essential trace elements from natural sources (fruits, vegetables, herbal teas etc.; Lugasi et al. 1998; Máday et al. 2000; Szentmihályi et al. 2000; Kocsis et al. 2004; Stefanovits-Bányai et al. 2005).

The main natural sources of antioxidant compounds, such as vitamins, polyphenols (flavonoids, anthocyanidins), as

Table 1. Element content (μg/g) in erythrocyte of healthy controls and sick patients*.

Elements	Control (N=50)	IBD patients (N=50)	Significance (P < 0.05)
Al	0.364 ± 0.313	0.443 ± 0.189	Not sign.
Ca	2.90 ± 2.25	0.975 ± 0.440	Sign.
Cu	0.029 ± 0.024	0.019 ± 0.005	Not sign.
Fe	24.48 ± 4.48	21.63 ± 2.24	Not sign.
Mg	18.28 ± 9.66	1.02 ± 0.24	Sign.
Mn	0.148 ± 0.234	0.024 ± 0.069	Not sign.
P	13.50 ± 4.14	10.86 ± 1.64	Not sign.
S	67.55 ± 15.90	56.11 ± 7.88	Not sign.
Zn	1.05 ± 0.480	0.776 ± 0.482	Sign.

*Data are presented as mean ± SD.

well as essential trace elements, are foodstuffs. Since fresh fruits and vegetables are known to contain these components in largest amount, in this study the nutritional habits of IBD patients and healthy controls were studied. For this purpose questionnaires were completed and blood samples were taken from patients and controls for evaluation and comparision.

Materials and Methods

Hydrogen peroxide was obtained from Sigma (St. Luis, MO, USA), nitric acid of 65% was purchased from Carlo Erba (Milano, Italy). Standard solutions for calibration were prepared from solutions of Spectrascan (Kolbotn, Norway) and High-Purity Standards (Charleston, SC, USA).

Survey

Randomly chosen 50 healthy Caucasian volunteers (Controls; 35 male and 15 female; ages between 25 and 47 years) and 50 IBD patients (25 male, 25 female; ages between 35 and 67 years) filled out questionnaires related to their nutritional habits in the consumption of foodstuffs. From the data obtained was that we could estimate the consumption of fresh fruits and vegetables and the nutritional components. The questions referred to both favored and non-favored fruits and vegetables, the frequency and amount of consumption, as well as the consumption of juice, wine, medicinal plant teas, teas, bread and kinds of bread. The questions were composed so that minimum one positive answer could be obtained. The basic principle of elaboration of the survey was published by Dörnyei et al. (2006).

Before filling out the questionnaires, blood samples were taken from the patients.

Preparation and evaluation of the questionnaire were made by permission of TUKEB 153/2000. The amount of elements consumed from foodstuffs was calculated based on the Table of Nutrients (Bíró and Lindner 1989). Since in our evaluation average values were used, therefore weigthed average was calculated for element intake/requirement from RDA and DRI values of male and female subjects (RDA 1989; DRI 2002). According to the calculation, and considering the ratio of male and female subjects (the two groups showed some differences: 70% males and 30% females in the control group, 48% males and 52% females in the IBD group), the following calculated average requirements (AR) were used in the control group: 4700 mg/day for K, 1000 mg/day for Ca, 390 mg/day for Mg, 12.4 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.2 mg/day for Mn. In the IBD group the following data were obtained: 4700 mg/day for K, 1000 mg/day for Ca, 360 mg/day for Mg, 14 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.1 mg/day for Mn.

Methods

The erythrocytes were separated and washed three times with isotonic NaCl solution. After washing and centrifugation procedures (10 min at 3000 rpm), the haemoglobin content of red blood cells was determined by the Haemisol Reagent Kit (HUMAN Vaccine Producing and Research Institute, Gödöllő, Hungary). The haemoglobin content was uniformly adjusted to 1 g/v%.

Inductively coupled plasma atomic emission spectrometry (ICP-AES, AtomScan 25, Thermo Jarrell Ash Co., Franklin, USA) was applied for the determination of Al, Ca, Cu, Fe, Mg, Mn, P, S, Zn concentrations in erythrocytes. Samples (1-2 ml) were digested with a mixture of HNO_3 (5 ml) and H_2O_2 (2 ml) in teflon vessels. After digestion, the samples were diluted to 10 ml with deionised water (Blázovics et al. 1999b). Since the washing of erythrocytes was performed with NaCl Na was not measured from the samples. In addition, since the intake of Na in the form of NaCl is generally higher than desired, the consumption of Na was not evaluated.

Routine laboratory parameters were controlled with the Roche/Hitachi Modular equipment followed by Ultra Sound investigations. The data obtained were published by Blázovics et al. (2006).

Means and standard deviations were calculated from the results. T-test was used for comparison of the results between the groups. Significance level was determined at $P < 0.05$.

Subjects

Blood samples of the above randomly chosen volunteers and IBD patients were evaluated. The patients were treated with 5-aminosalycilic acid (5-ASA, WHO proposed therapy). Patients with inactive UC and CD were treated with 5-ASA and 5-ASA plus the immunosuppressor azathioprine, respectively. Patients with moderate UC and CD were given 5-ASA plus a local steroid, and 5-ASA plus azathioprine plus the local antibiotic metronidazole, respectively. Patients with severe UC were treated with 5-ASA plus a local steroid and/or systemic steroid. Patients with severe CD received combined therapy with (metronidazole or ciprofloxacin) plus elementary

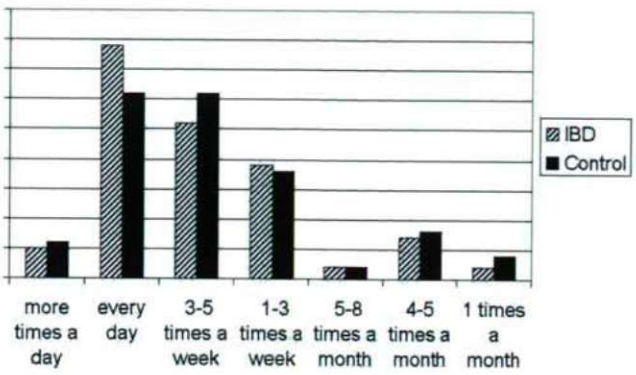

Figure 1. Frequency of fruit consumption.

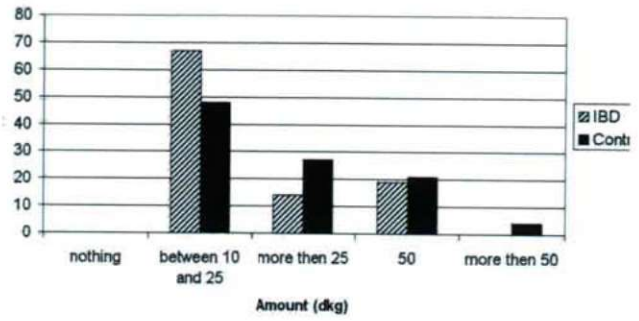

Figure 2. Amount of fruit consumed.

diet and steroid (local and/or systemic) plus antibiotics. The study was approved by the Regional Committee of Science and Research Ethics, Semmelweis University. Permission number: TUKEB 24/1996, renewed in 2000.

Although our earlier investigations showed that in both males and females the redox parameters of plasma and erythrocytes change according to the severity of the disease independently of the antioxidant therapy (Blázovics et al. 2000; Szentmihályi et al. 2000), in this study the severity of the disease was not examined. This survey examined the nutritional habits of patients compared to the controls independently of the momentary state of the patient. All appreciable data of patients were elaborated from randomly chosen volunteers.

Results

Element homeostasis depends on many factors e.g. the amount of food, nutritional habits, the form of element consumed, the element state of the body. Therefore, the differences in the element content of erythrocytes of healthy controls and IBD patients were monitored first. The element content of erythrocytes is summarized in Table 1. The high value of standard deviations in the control group confirms that the group was chosen randomly from healthy people and some of these "healthy controls" may suffer from other metabolic diseases or latent deficiency state. In spite of this the concentration of Ca, Mg and Zn in the group of IBD patients was significantly lowered at $P<0.05$ calculated by the t-test.

Consumption of fruits

The frequency of fruit consumption and the amount of fruit consumed by the IBD patients slightly differ from the data of controls (Figs. 1 and 2). Although patients generally eat fruit more frequently than healthy people, they eat smaller amounts at a time. The fruits most favoured by IBD patients are peaches (93%), apples (80%), cherries (78%), watermelons (78%), bananas (76%), and apricots (62%). In spite of recommendations, some fruits, with small seeds, which are not favorable for patients, are consumed relatively frequently: strawberries (78%), raspberries (72%), white grapes (62%). Healthy people also like peaches (80%), apples (76%), bananas (60%), cherries (60%), strawberries (60%), white grapes (58%), apricots (58%), water-melons (54%), raspberries (54%), although other fruits are also under consumption: oranges (60%) and pears (54%). The daily amount of fresh fruit was found to be 0.15 kg for IBD patients and 0.17 kg for controls.

Almost totally neglected fruits by the two groups are avocado, blackberries, blueberries, cornel, currant species, gooseberries, maracuja, naseberries, quince apples, quince pears, rose-hips, wild plums. Some of the fruits, which are grown in Hungary, e.g. currant species, naseberries, quince apples, quince pears, rose-hips, wild plums are consumed almost only by small garden owners.

Cherries, walnuts, strawberries, raspberries, pears and plums cause complaints in about one-fifth of IBD patients, while bananas, walnuts, white grapes, apples and plums cause complaints in the controls.

Table 2 shows the daily element intake of fruits for controls and IBD patients calculated from the data of questionnaires. As can be seen, there is only a small difference between the element intake of the two groups in absolute value and in percentage related to AR calculated by RDA and DRI values (1989; 2002). Element consumption ranges between wide intervals depending on nutritional habits, e.g. IBD patients consume K between 368 and 1840 mg. Minimum consumption for an individual seems to be the same in both groups, although the maximum value shifts towards higher values in the case of controls, owing to nutritional habits. In all cases average element intake was found to be below 50% in IBD patients, while it was between 14 and 55% for the control group.

Consumption of vegetables

The frequency of vegetable consumption by controls and IBD patients shows only a slight difference (Fig. 3). IBD

Table 2. Element intake a day from fresh fruits according to the nutritional habits in Hungary.

	K	Ca	Mg	Fe	P	Cu	Zn	Mn
Control (n=50)								
Minimum intake for an individual (mg)	368	75	59	1.2	80	0.1	0.7	0.4
Maximum intake for an individual (mg)	2940	597	469	9.4	642	1.1	5.3	3.1
Weighted mean for the group (mg)	1136	231	181	3.6	248	0.44	2.1	1.2
Minimum intake refer to AR (%)	7.8	7.4	15.0	9.5	10.0	15.8	4.4	17.7
Maximum intake refer to AR (%)	62.5	59.7	120	76.1	80.2	127	35.5	142
Average intake refer to AR (%)	24.2	23.1	46.5	29.4	31.0	48.9	13.7	54.8
IBD patients (n=42)								
Minimum intake for an individual (mg)	368	75	59	1.2	80	0.1	0.7	0.4
Maximum intake for an individual (mg)	1840	373	293	5.9	401	0.7	3.3	2.0
Weighted mean for the group (mg)	948	192	151	3.1	207	0.37	1.7	1.0
Minimum intake refer to AR (%)	7.8	7.5	16.3	8.4	10.0	15.8	4.4	18.6
Maximum intake refer to AR (%)	39.0	37.3	81.5	42.1	50.2	79.2	22.2	92.9
Average intake refer to AR (%)	20.2	19.3	42.0	21.7	25.9	40.8	11.4	47.9

AR average requirement (Calculated data according to the DRI, RDA data and the ratio of male and female subjects: 70% males and 30% females in the control group, 48% males and 52% females in the IBD group. The following AR values were used in the control group: 4700 mg/day for K, 1000 mg/day for Ca, 390 mg/day for Mg, 12.4 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.2 mg/day for Mn. In the IBD group the following data were obtained: 4700 mg/day for K, 1000 mg/day for Ca, 360 mg/day for Mg, 14 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.1 mg/day for Mn.)

patients eat vegetables more rarely than healthy people. The most favoured vegetables of IBD patients are potatoes (95%), tomatoes (90%), cucumbers (81%), carrots (80%), green peppers (76%), lettuce (76%), onions (76%), cauliflowers (71%), green pease (71%), beetroots (71%), kale (67%) and garlic (62%). Of these vegetables garlic and kale may cause complaints sometimes for IBD patients and therefore, some of these patients never eat kale. Although cauliflowers, cucumbers, green peppers and beetroots are not recommended they are much favoured and consumed by IBD patients. Healthy controls also like potatoes (94%), cauliflowers (94%), tomatoes (78%), lettuce (76%), carrots (72%), green peppers (72%) and maize (80%). The total amount of vegetables to be consumed a day is 0.25 kg for IBD patients and 0.30 kg for controls.

Some vegetable almost never consumed s are those rarely available in the market or originating from abroad: brussels sprouts, chard, parsnips, asparagus, rheum and pumpkins. Vegetables cause complaints more frequently in IBD patients than in controls, although the vegatables causing problems are the same in both groups: kale, cabbage, kohlrabies and maize.

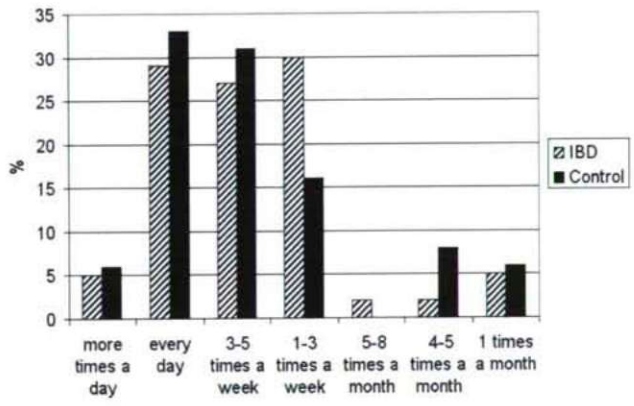

Figure 3. Frequency of vegetable consumption.

Table 3 shows the daily element intake of vegetable for IBD patients and controls calculated on the basis of questionnaires. Similarly to the element intake from fruits, only a slight difference could be observed in the element intake between patients and controls in absolute value and in percentage related to AR. Minimum and maximum consumption seems to be the same in both groups. Average element intake ranges between 4.0 and 17.7% in IBD patients, while it changes between 4.2 and 18.3% for the controls.

Total consumption of fruits and vegetables

Based on the evaluation of the survey, element intake by vegetables and fruits covers only a part of the daily requirements (Table 4). This value ranges between 15-60% for IBD patients and 18-66% in the controls. Although plants are known to be good sources of K and Mn, the intake of K does not reach 30% in IBD group. The low Zn intake (15% of the requirement in general) and Ca (24% of the requirement in general) in the case of IBD patients is a serious problem. The main sources of Ca are dairy products and of Zn are meats, although patients hardly eat any dairy products and the consumption of meat is also reduced mainly because of intolerance.

Discussion

Hungarian people consumes essential elements in relatively small amount as verified by the significantly low element concentration of Ca, Mg and Zn in the erytrocytes of IBD

Table 3. Element intake a day from vegetables according to the nutritional habits in Hungary.

	K	Ca	Mg	Fe	P	Cu	Zn	Mn
Control (n=50)								
Minimum intake for an individual (mg)	19	2.21	2.13	0.05	3.84	0.01	0.03	0.01
Maximum intake for an individual (mg)	1665	189	183	3.87	329	0.62	2.34	0.97
Weighted mean for the group (mg)	444	51	49	1.0	88	0.165	0.624	0.26
Minimum intake refer to AR (%)	0.4	0.2	0.5	0.4	0.5	0.8	0.2	0.5
Maximum intake refer to AR (%)	35.4	18.9	46.9	31.2	41.1	68.7	15.6	44.2
Average intake refer to AR (%)	9.4	5.1	12.5	8.3	10.9	18.3	4.2	11.8
IBD patients (n=42)								
Minimum intake for an individual (mg)	19	2.21	2.13	0.05	3.84	0.01	0.03	0.01
Maximum intake for an individual (mg)	1665	189	183	3.87	329	0.62	2.34	0.97
Weighted mean for the group (mg)	429	49	47	0.99	85	0.159	0.60	0.25
Minimum intake refer to AR (%)	0.4	0.2	0.6	0.3	0.5	0.8	0.2	0.4
Maximum intake refer to AR (%)	35.4	18.9	50.8	27.6	41.1	68.7	15.6	46.3
Average intake refer to AR (%)	9.1	4.9	13.1	7.1	10.6	17.7	4.0	11.9

AR average requirement AR (Calculated data according to the DRI, RDA data and the ratio of male and female subjects: 70% males and 30% females in the control group, 48% males and 52% females in the IBD group. The following AR values were used in the control group: 4700 mg/day for K, 1000 mg/day for Ca, 390 mg/day for Mg, 12.4 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.2 mg/day for Mn. In the IBD group the following data were obtained: 4700 mg/day for K, 1000 mg/day for Ca, 360 mg/day for Mg, 14 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.1 mg/day for Mn.)

Table 4. Element intake a day from fresh vegetables and fruits according to the survey of nutritional habits in Hungary.

	K	Ca	Mg	Fe	P	Cu	Zn	Mn
Control (n=50)								
Average intake (mg)	1580	282	230	4.6	336	0.6	2.72	1.46
Average intake refer to AR (%)	34	28	59	37	42	67	18	66
IBD patients (n=42)								
Average intake (mg)	1376	241	198	4	292	0.53	2.3	1.25
Average intake refer to AR (%)	29	24	55	29	36	59	15	60

AR average requirement (Calculated data according to the DRI, RDA data and the ratio of male and female subjects: 70% males and 30% females in the control group, 48% males and 52% females in the IBD group. The following AR values were used in the control group: 4700 mg/day for K, 1000 mg/day for Ca, 390 mg/day for Mg, 12.4 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.2 mg/day for Mn. In the IBD group the following data were obtained: 4700 mg/day for K, 1000 mg/day for Ca, 360 mg/day for Mg, 14 mg/day for Fe, 800 mg/day for P, 0.9 mg/day for Cu, 15 mg/day for Zn and 2.1 mg/day for Mn.)

patients compared to the controls and according to the survey by the low element intake of Zn, Ca, K and Fe from fruits and vegetables. Similar results were published by Geerling and co-authors, namely lowered Ca and P intake was observed in IBD patients (Geerling et al. 2000). Non-favorable nutritional habits may contribute to the development of IBD (Tragnone et al. 1995; Mahmud and Weir 2001) and in IBD patients macro- and microelement deficiency may frequently occur (20-90% of the patients) (Gasche et al. 2004; Fehér and Kovács 2007). Decreased oral element intake, malabsorption and increased intestinal bleeding are the major causes of deficiency. According to the literature, mainly Ca, Mg, Fe, Se and Zn deficiency occurs in IBD patients, although the rate of deficiency depends on the severity of the disease as well (Galland 1988; Rennem et al. 1998; Schoon et al. 1999; Gasche et al. 2004; Vijverman et al. 2006). Probably the most serious problem is the Zn deficiency, since the human body does not store Zn and a high amount of Zn is lost with the faeces, urine and through the skin by sweat (Tapiero and Tew 2003). Zn deficiency may cause diarrhea (Saxena et al. 1993), although the deficiency may be treated by supplementation of elements. High doses of inorganic metal compounds, e.g. Fe supplementation with inorganic $FeSO_4$ may be dangerous because of the increased oxidative stress and cancer risk (Seril et al. 2006). Toxic or large amount of metal elements e.g. Mn, Fe, Al may induce the signal transduction process via activation of mitogen-activated protein kinases (Kaneki et al. 2004; Valko et al. 2006). Administration of high amount of Zn may alter Fe metabolism (Blázovics et al. 2004b). Mg deficiency may cause transient increase in intracellular Ca level, which induces the production of pro-oxidant cytokines (interleukin-1, -6 and -8, tumor necrosis factor-α and -β), different growth factors (EGF-α, TGF-β, NFGF, FGF, PDGF), interferon-α and $-\gamma$. The increase in intracellular Mg level inhibits the production of pro-oxidant cytokines via the activation of protein phosphatases (Caddell 2000). Therefore the achievement of optimal metal element level is essential for the proper function of cells and body.

There is no doubt, element supplementation is very important for IBD patients. Since the long-term supplementary diet may cause several unfavorable effects, medical control is essential throughout the treatment. The consumption of fresh fruits and vegetables should be recommended and patients must be informed of plant foodstuffs most favorable from nutritional aspects. For IBD patients most recommended fruits are apples, peaches, apricots, bananas, nectarines and water-melons. According to the survey the patients do eat these fruits but in relatively small amount. At the same time some valuable fruits rich in K, Ca, Mg and Zn, e.g. rose-hips, gooseberries, raspberries, white grapes, currants, walnuts, hazel nuts and almonds are contra-indicated for patients. The situation is similar for vegetables, since the vegetables most rich in elements, e.g. spinach, wood sorrel, mushrooms, garlic are not recommended (Kocsis et al. 2004; Fehér and Kovács 2007). Malnutrition is aggravated by low consumption of milk and meat products.

Plant foodstuffs contain essential mineral elements in the form of organic compounds as well as antioxidant polyphenols and vitamins. In Hungary, as well as in other countries throughout the world, some diseases (e.g. cardiac, vascular diseases and cancer) associated with nutritional habits and life style are responsible for the high rate of mortality (75%). Therefore, several surveys have been made to estimate or determine the consumption of vegetables and fruits or the intake of vitamins and mineral elements. Based on commercial data in Hungary, the yearly ingestion/person of fruits and vegetables was 211.4 kg in 2004, from which the rate of vegetables was 117.7 kg (with 68 kg potatoes). According to the Hungarian Central Statistical Office, the consumption of vegetables and fruits was 195 kg in 2004 (110 kg vegetables and 85 kg fruits) (Hungarian Central Statistical Office 2005). Our survey shows that the consumption of plant foodstuffs (fruits and vegetables) amount to 146 kg/year (0.4 kg/day) for IBD patients and 171.6 kg/year (0.47 kg/day) for controls. The difference may be due to the amount of potatoes eaten as chips or fried potatoes (which were omitted by the participants of survey).

Conclusion

The mineral element imbalance was found to be higher in IBD patients, which probably contributes to the deficiency states. Since both groups, patients and controls are on a similar diet, the low element intake may cause latent element deficiency in healthy people as well, which may enhance the risk of metabolic disorders.

References

Bhaskar L, Ramakrishna BS (1995) Balasubramanian KA. Colonic mucosal antioxidant enzymes and lipid peroxide levels in normal subjects and patients with ulcerative colitis. J Gastroenterol Hepatol 10:140.

Bíró G, Lindner K (1995) Table of Nutrients, Budapest, Medicina Kiadó.

Blázovics A, Kovács Á, Lugasi A, Hagymási K, Bíró L, Fehér J (1999a) Antioxidant defense in erythrocytes and plasma of patients with active and quiescent Crohn's disease and ulcerative colitis: A chemiluminescence study. Clin Chem 45:895-896.

Blázovics A, Kovács Á, Lugasi A, Hagymási K, Bíró L, Fehér J (1999b) Antioxidant defence in erythrocytes and plasma of patients with active and quiescent Crohn's disease and ulcerative colitis: A chemiluminescent study. Clin Chem 45:895-896.

Blázovics A, Kovács Á, Lugasi A, Hagymási K, Bíró L, Fehér J (2000) Total scavenger capacity of erythrocytes and plasma is a good predictive factor in inflammatory bowel diseases. Curr Top Biophys 24:19-28.

Blázovics A, Szentmihályi K, Prónai L, Hagymási K, Lugasi A, Kovács Á, Fehér J (2004a) Redox-homeosztázis gyulladásos bélbetegségekben (Redox homeostasis in inflammatory bowel diseases). Orvosi Hetilap 145:1459-1466.

Blázovics A, Szentmihályi K, Vinkler P, Kovács Á (2004b) Zn overdose may cause disturbance in iron metabolism in inactive inflammatory bowel diseases. Trace Elem Electrol 21:240-247.

Blázovics A, Kovács Á, Székely E, Lugasi A (2006). Effect of product containing black radish (Raphanus sativus L. var. niger) on redox-homoestasis of IBD patients (Hungarian). LAM 16:234-240.

Bruwer M, Scmidt KW, Metz KA, Kriegistein CF, Senninger N, Schumann G (2001) Increased expression of metallothionein in inflammatory bowel disease. Inflamm Res 50:289-293.

Caddell JL (2000) Geriatric cachexia: a role for magnesium deficiency as well as for cytokines? Am Soc Clin Nutr 71:851-852.

Dörnyei O, Kovács Á, Székely E, Dinya E, Blázovics A (2006) Antioxidánsok fogyasztására vonatkozó felmérések gyulladásos bélbetegségekben. (Surveys concerning the consumption of nutritional antioxidants in cases of inflammatory bowel diseases) Orvosi Hetilap 147(23):29-37.

DRI (2002) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Food and Nutritional Board, Institute of Medicine. Boston: National Academic Press.

Fehér J, Kovács Á (2007) Gyulladásos bélbetegségek, Újdonságok a szemléletben (Inflammatory bowel diseases, Novelties in the view) Budapest: Intermedicons Kft.

Galland L (1988) Magnesium and inflammatory bowel disease. Magnesium 2:78-83.

Gasche C, Lomer MC, Cavill I, Weiss G (2004) Iron, anaemia and inflammatory bowel diseases. Gut 53:1190-1197.

Geerling BJ, Badart-Smook A, Stockbrugger RW, Brummer RJM (2000) Comprehensive nutritional status in recently diagnosed patients with inflammatory bowel disease compared with population controls. Eur J Clin Nutr 54:514-521.

Goldschmid S, Graham M (1989) Trace element deficiencies in inflammatory bowel disease. Gastroenterol Clin North Am 18:579-587.

Hungarian Central Statistical Office (2005) Household budget survey report. Budapest.

Kaneki H, Ishibashi K, Kurokawa M, Fujieda M, Kiriu M, Mizuochi S, Ide H(2004) Mechanism underlying the aluminium-induced stimulation of bone nodule formation by rat calvarial osteoblasts. J Health Sci 50: 47-57.

Kocsis I, Szentmihályi K, Rapavi E, Hubay M, Kéry Á, Stefanovits-Bányai É, et al. (2004) Effect of *Cichorium intybus* (L.) on the redox balance and metal ion content of fatty liver in hyperlipidemic rats. Trace Elem Electrol 21:262-268.

Kudrin AV (2000) Trace elements in regulation of NF-κB activity. J Trace Elem Med Biol 2000; 14: 129-42.Sheikh MS, Fornace AJJr. Death and decoy receptors and p53-mediated apoptosis. Leukemia 14:1509-13.

Lugasi A, Dworschák E, Blázovics A, Kéry Á (1998) Antioxidant and free radical scavenging properties of squeezed juice from black radish (Raphanus sativus L. var, niger) root. Phytother Res 12:502-506.

Máday E, Szentmihályi K, Then M, Szőke É (2000) Mineral element content of chamomile. Acta Aliment 29:51-57.

Mahmud N, Weir DG (2001) The urban diet and Crohn's disease: is there a relationship? Eur J Gastroenterol Hepatol 13:93-95.

Rannem T, Ladefoged K, Hylander E, Hegnhoj J, Staun M (1998) Selenium

depletion in patients with gastrointestinal diseases: Are there any predictive factors? Scand J Gastroenterol 33:1057-1061.

RDA (1989) Recommended Dietary Allowances. 10th ed. NY, Washington DC: National Academy Press.

Saxena R, Bedwal RS, Mathur RS (1993) Histopathology of the testes of zinc-deficient albino-rats. Trace Elem Med 10:177-180.

Schoon EJ, Wolffenbuttel BHR, Stockbrugger RW (1999) Osteoporosis as a risk in inflammatory bowel disease. Drugs Today 35:17-28.

Sheikh MS, Fornace AJJr (2000) Death and decoy receptors and p53-mediated apoptosis. Leukemia 14:1509-13.

Seril DN, Liao J, West AB, Yang GY (2006) High-iron diet: Foe or feat in ulcerative colitis and ulcerative colitis-associated carcinogenesis. J Clin Gastroenterol 40:391-397.

Stefanovits-Bányai É, Szentmihályi K, Hegedűs A, Koczka N, Váli L, Taba G, Blázovics A (2005) Metal ion and antioxidant alterations in leaves between different genders of Ginkgo biloba L. (Ginkgoaceae), Life Sci 78:1049-56.

Sturniolo GC, Mestriner C, Licis PE, D'Odorico A, Venturi C, Irato P, Cecchetto A, Tropea A, Longo G, D'Inca R (1998) Altered plasma and mucosal concentrations of trace elements and antioxidants in active ulcerative colitis. Scand J Gastroenterol 33:644-649.

Szentmihályi K, Then M (2000a) Teas of Equiseti herba, Myrtilli folium and Salviae folium. Acta Aliment 29:43-49.

Szentmihályi K, Blázovics A, Kocsis I, Fehér E, Lakatos B, Vinkler P (2000b) The effect of fat rich diet and alcohol on ion concentration in bile fluid in rats. Acta Aliment Hung 29:359-366.

Tapiero H, Tew K (2003) Trace elements in human physiology and pathology: zinc and metallothioneins. Biomed Pharmacother 57:399-411.

Tragnone A, Valpiani D, Miglio F, Elmi G, Bazzocchi G, Pipitone E, Lanfrachi A (1995) Dietary habits as risk factors for inflammatory bowel disease. Eur J Gastroenterol Hepatol 7:47-51.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress induced cancer. Chem-Biol Interact 160:1-40.

Vijverman A, Pirnt P, Belaiche J, Louise E (2006) Evolution of prevalence and characteristics of anemia in inflammatory bowel diseases between 1993 and 2003. Acta Gastro-Enterol Belg 69:1-4.

Volume 53(Suppl.1): 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Prooxidant mechanisms of selenium toxicity – a review

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ABSTRACT Selenium is an essential trace element in living organisms as integral part of seleno-enzymes. However, excess amount of selenium is toxic for so-called non-accumulator plants, animals and humans. The toxicity for plants depends on the capacity of synthesis of non-protein amino acids and also their volatilization in the form of dimethylselenide, while in animals on the rate of methylation and its excretion. In vitro studies showed that there are selenium-resistant animal and human cell lines which showed altered selenium uptake. Exact mechanism of selenium toxicity remains unclear but there are many data about its prooxidant effect particularly in the form of selenite, while selenomethionine and selenocysteine are less toxic. Inorganic forms of selenium reacts with tissue thiols, such as glutathione to form seleno-trisulphides and those are reacting with other thiols to generate oxygen free radicals, such as superoxide anion. Organic diselenides are converted into selenols in presence of thiols which also results oxygen free radical generation. Another free radical hypothesis of selenium toxicosis is based on the methyl-selenide formation, which also results superoxide radicals and induce oxidative stress. Besides free radical formation selenium can have inhibitory effects on thiol proteins, for instance those which have antioxidant affect.
Acta Biol Szeged 53(Suppl.1): (2009)

KEY WORDS

free radicals
selenium
prooxidant

Trace elements are essential for maintenance of health, growth, and many biochemical-physiological functions of animals and humans (Scott et al. 1982). Among these essential trace elements selenium was discovered by Berzelius in 1818, but its biological significance was not recognized until it was identified as the toxic agent associated with 'alkali disease', now termed selenosis, in the United States in 1856 (Franke 1934). Selenium was known as toxic material up to 1957 when Schwarz and Foltz found as essential element for prevention of liver necrosis in vitamin E deficient rats (Schwarz and Foltz 1957) and later the discovery that selenium is the integral part of selenium-dependent glutathione peroxidase enyzmes demonstrated a biochemical role for this essential trace element and provided a tool for monitoring its status in animals and humans (Rotruck et al. 1973).

Selenoamino acids, selenomethionine, selenocysteine and selenocystine, are the primary sources of naturally-occurring selenium in plant-based (Burk 1976) and meat-based (Levander 1986) feed and food ingredients. The selenoamino acids are bound in protein, principally as selenomethionine and selenocysteine and constitute 50 to 80% of the total selenium in plants (Butler and Peterson 1967) and in selenium enriched yeast (Kelly and Power 1995). Animals can not synthesize selenomethionine, the primary selenoamino acid, directly from the selenite or selenate forms of inorganic selenium (Sunde 1990). However, selenocysteine can be found in the body of animals fed inorganic selenium such as selenite and selenate. The presence of selenocysteine is due to synthesis of glutathione peroxidase and other selenoproteins in which the selenocysteine is incorporated. The synthesis of selenocysteine involves a unique process in which selenide is phosphorylated by selenophosphate synthetase to selenophosphate. The selenophosphate is made available to a unique seryl-tRNA^{SEC} that is recognized by selenocysteine synthetase. The selenocysteine synthetase converts seryl-tRNA^{SEC} to selenocysteyl-tRNA^{SEC} that allows insertion of selenocysteine into a peptide chain. The selenocysteine insertion also requires a specific mRNA, an elongation factor, GTP, and the selenocysteine insertion sequence that all interact at the ribosome to read the UGA selenocysteine codon (Low and Berry 1996). Selenomethionine can not be synthesized de novo by animals, therefore it has to supply with feed ingredients and it is easily converted to selenocysteine via cystothionase (Esaki et al. 1981). Selenocysteine can be substituted for cysteine in many proteins, but it is not incorporated directly into specific selenoproteins (Sunde 1990). Selenocysteine is the pivotal amino acid in the synthesis of selenium-dependent cytosolic glutathione peroxidase (Rotruck et al. 1973), but only about 30% of the body's selenium is incorporated into that enzyme. About 70% of total selenium is incorporated into the other – recently known about 30-50 – selenoproteins in animals and humans (Behne and Kyriakopoulos 2001; Kryukov et al. 2003).

Accepted June 19, 2009
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Table 1. Ability of selenium compounds to generate superoxide in vitro (adapted from Surai 2006).

Superoxide produced	Superoxide not produced
Selenite	Selenomethionine
Selenium dioxide	Selenate
Selenocystine	Elemental selenium
Diselenodipropionate	Selenobetaine
Diphenylselenide	Potassium-selenocyanate

Selenium toxicosis in living organisms

Selenium is toxic for prokaryotes, such as bacteria or algae, however, long-term continous selenium exposition results selection of selenium resistant strains (Burton et al. 1987). Selenium resistance depends on the capacity of reduction from selenate or selenite to selenide (Oremland et al. 1994).

Among plant species there are accumulators (e.g. Astragallus, Conopsis, Xylorrhiza, Oonopsis, Stanleya spp.) which are highly tolerant of selenium (Schrauzer 2003), and non-accumulators which are poisoned by selenium (Smith and Watkinson 1984). The toxicity of selenate and selenite to non-accumulator plants can be attributed to combination of different factors such as the chemical form of selenium (selenate or selenite) and conversion of selenium anions into organic forms or organic metabolites (selenocysteine and selenomethionine). These selenoamino acids act as analogues of essential sulphur compounds, but the physical and chemical differences between selenium and sulphur will result in small, but significant, changes in the biological properties of a selenium-substituted protein (Brown and Shrift 2008). Selenium-tolerant accumulator plants differ from sensitive species, because those contain large quantities of non-protein amino acids, such as seleno-methyl-selenocysteine and seleno-cystathionine or γ-glutamyl-seleno-methyl-cysteine, which are bound selenium and decrease its phytotoxic effect (Pyrzynska 2002). However, these selenoamino acids are rarely detected in non-accumulator plants. In addition, selenium is kept from entering proteins so that the selenium levels in proteins of accumulator plants is significantly lower than the levels in selenium-sensitive plants (Brown and Shrift 2008). Plants also volatilized selenium to dimethylselenide, and volatilization increased linearly with external selenium concentration (De Souza et al. 1998).

Selenium can be toxic for all animals, such as invertebrates (US EPA 1987), fishes (Balogh et al. 2002), amphibians and reptiles (Birge 1978), birds (Green and Albers 1997), mammals (Goehring 1984; McDowell 1997; ATSDR 2003) and humans (Yang et al.1983; Moeasgaard and Morrill 2002; ATSDR 2003) depending on the dose and duration of intake, and also on its chemical form. Tolerance for selenium toxicity depends on, among other factors, the rate of excretion, and selenium excretion depends on the rate of methylation of selenium as was found in fishes (Hilton et al. 1982).

In vitro studies showed that toxic doses of selenium provoke cancer (Schrauzer and Ishmael 1974) but there are also selenium-resistant cell lines which showed altered selenium uptake and intracellular glutathione concentrations. The possible mode of resistance is based on two 72 kDa selenium-labelling proteins which are important for the altered selenium uptake (Wu et al. 1995).

In contrary selenium compounds that form the methylselenide anion (selenol) have been shown to induce cellular apotosis even in tumour cells, and one selenium compound, selenium-methylseleno-cysteine, induced apotosis in cancer cells through activation of capsases, a likely mechanism for other selenium compounds that also induce apotosis (Ganther 1999; Spallholz 2001).

Prooxidant mechanism of selenium toxicosis

The molecular mechanism of selenium toxicity remains unclear but there is an increasing database that shows the pro-oxidant effect of excess selenium, particularly in the form of selenite (Hafeman et al. 1974; Csallany and Menken 1986, Spallholz 1997; Terada et al. 1999; Raisbeck 2000). However, selenomethionine and selenocysteine, particularly their L-isomers are less toxic than sodium-selenite (Spallholz 1994).

Selenium compounds have different abilities to generate superoxide in vitro as shown in Table 1. Inorganic forms of selenium appear to react with tissue thiols, such as glutathione (Garberg et al. 1988) to form seleno-trisulphides and those are reacting with other thiols to generate oxygen free radicals, such as superoxide anion ($O_2^{\cdot-}$) by redox catalysis (Seko et al. 1989). Selenite reacts with glutathione endogenously in cells or extracellularly causes toxicity by the formation of superoxide and elemental selenium (Seko et al. 1989; Spallholz 1994; Seko and Imura 1997) according to the following reaction scheme (Shen et al 2000).

$SeO_3^{2-} + 4GSH \rightarrow GSSeSG + GSSG$

$GSSeSG + GSH \rightarrow GSSeH + GSSG$

$GSSeH + GSH \rightarrow H_2Se + GSSG$

$H_2Se + O_2 \rightarrow Se^0 + O_2^{\cdot-}$

Organic diselenides (e.g. selenocystine and selenocystamine are converted into selenols (RSeH) in presence of thiols which also results oxygen free radical generation during further reductions catalyzes the formation of superoxide under aerobic conditions in the presence of thiol; this reaction could play a role in the toxicity of diselenides and alkylselenols (Chaudiere et al. 1992).

Those selenium compounds such as elemental selenium (Gao et al. 2000), selenates and seleno-ethers (RSeR), that do not readily form a selenide (RSe-) anion, or selenoenzymes, where selenium is sequestered, therefore do not react with thiols, are non toxic or only after reduction to selenite or selenol (Spallholz 1994).

Another free radical hypothesis of selenium toxicosis is also described (Spallholz and Hoffman 2002). It is based on

the methyl-selenide formation, which also results superoxide radicals and at least oxidative stress. Excess selenium in the form of selenocysteine inhibits the methylation of selenium and increases the amount of intermediary metabolite, hydrogen-selenide, which can also be toxic (Ganther 1979).

Besides free radical formation selenium can have inhibitory effects on thiol proteins, for instance those which have antioxidant affect, by modification via (1) formation of S-Se-S (selenotrisulfides) and S-Se (selenylsulfide) bonds, (2) catalysis of S-S (disulfide bonds) with no incorporation of selenium in the protein, and (3) formation of Se-Se diselenides (Ganther 1999). This catalytic reaction of selenium compounds with thiols likely accounts for selenium toxicity to cells ex vivo and in vivo where the major glutathione producing organ, the liver, is also the major target organ of selenium toxicity. Selenium also inhibits several thiol-containing enzymes, such as methionine-adenosyltransferase, succinate-dehydrogenase, lactate-dehydrogenase and NADP+-isocitrate-dehydrogenase (Nebbia et al. 1990).

The prooxidant activity of selenium may also account for cellular apoptosis and may provide a useful pharmaceutical application for selenium compounds as antibacterial, antiviral, antifungal and anticancer agents (Spallholz 1997; Nilsonne et al. 2006). However, various human carcinoma cell lines (e.g. breast, hepatoma, neuroblastoma and colon carcinoma) showed different sensitivity to selenium derivatives, such as methyl-L-selenocysteine or selenomethionine (Jariwalla et al. 2009).

The produced free radicals are involved in uncontrolled chain reactions, which affect biomolecules, primarily phospholipids, causing lipid peroxidation (Halliwell and Gutteridge 2007). Selenium toxicosis (acut or chronical) turns up when the level of oxidative damage exceeds the capacity of antioxidant defense system, or exceeds the ability of the organism to build the potencially reactive selenocompounds in selenoproteins, or convert them to non-reactive forms (Yan and Spallholz 1993). Selenium toxicosis causes DNA damage (Kelly et al. 1998) by generating 8-hydroxyguanosine DNA adducts (Kim et al. 2004) and lipid peroxidation, and as an effect of the oxidative stress, membranes (e.g. cell-organelle membranes) loose their integrity thus lysosomal enzymes can leak out of them causing serious necrotic damage in tissues (Mézes and Matkovics 1986; Mézes and Sályi 1994; Balogh et al. 2007). Toxic dose of selenium in form of selenite (6 mg kg^{-1} body weight per day for 12 days) causes increase the formation of malondialdehyde, as marker of lipid peroxidation, also decreases the amount of reduced glutathione and activities of antioxidant enzymes catalase and superoxide dismutase, but up-regulated glutathione peroxidase in liver of mice (Zhang et al. 2005).

Toxicokinetic studies with chicken embryo showed that toxic doses of selenium reduced the level of lipid peroxides significantly both in hepatic and brain tissues at early hours after exposure and it was gradually increased thereafter to normal levels later. Further, the effect of selenium on some of the antioxidant enzymes likes glutathione peroxidase, glutathione transferase and superoxide dismutase were increased at 6 h post treatment but glutathione levels were reduced in both hepatic and brain tissues (Padmaja et al. 1997). In three weeks-old chicken early period of exposure of excess amount of selenite or selenomethionine increased the level of lipid peroxides and also glutathione peroxidase activity (Balogh et al. 2007).

Detoxification of selenium

Methylation of selenium by both plants (Terry et al. 2000) and animals (Hasegawa et al. 1996) serves to detoxify selenium by generating methylselenides, however excess amount of selenium in the form of selenocysteine decreases of the methylation of selenium (Spallholz and Hoffman 2002). Alternatively, full reduction of Se to elemental selenium (Se^0) as done by some bacteria and the formation of heavy metal selenides such as Ag_2Se or Hg_2Se, results in a non-catalytic non-toxic form of selenium. This catalytic prooxidant attribute of some selenium compounds appears to account for its toxicity when such activity exceeds plant and animal methylation reactions and antioxidant defenses. The excess selenium alternatively can be catabolized into hydrogen selenide and secreted in breath or into trimethyl-selenonium ion and secreted through urine (Ip 1998).

References

ATSDR (2003) Toxicological profile for selenium. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, pp. 1-457.

Balogh K, Elbaraasi H, Mézes M (2002) Selenium toxicity in fishes (In Hungarian). Halászat 95:30-33.

Balogh K, Weber M, Erdélyi M, Mézes M (2007) Investigation of lipid peroxide and glutathione redox status of chicken concerning on high dietary selenium intake. Acta Biol Hung 58:269-279.

Birge WJ (1978) Aquatic toxicology of trace elements of coal and fly ash. In Thorp JH, Gibbons JW eds., Energy and Environmental Stress in Aquatic Systems. Department of Energy Symposium Series 48:219-240.

Behne D, Kyriakoupulos A (2001) Mammalian selenium containing proteins. Annu Rev Nutr 21:453-473.

Brown, TA, Shrift A (2008) Selenium toxicity and tolerance in higher plants. Biol Rev 57:59-84.

Burk RF (1976) Selenium in man. In Prasad AS, Oberleas D eds., Trace Elements in Human Health and Disease. Academic Press, New York, 105-133.

Burton GA Jr, Giddings TH, DeBrine P, Fall R (1987) High incidence of selenite-resistant bacteria from a site polluted with selenium. Appl Environ Microbiol 53:185-188.

Butler GW, Peterson PJ (1967) Uptake and metabolism of inorganic forms of selenium-75 by Spiroldela oligorrhiza. Aust J Biol Sci 20:77-81.

Chaudiere J, Courtin O, Leclaire J (1992) Glutathione oxidase activity of selenocystamine: A mechanistic study. Arch Biochem Biophys 296:328-336.

Csallany AS, Menken BZ (1986) Effect of dietary selenite on hepatic organic solvent-soluble lipofuscin pigments. J Am Coll Toxicol 5:79-85.

De Souza MP, Pilon-Smits EAH, Mel Lytle C, Hwang S, Tai J, Honma TSU, Yeh L, Terry N (1998) Rate-limiting steps in selenium assimilation and

volatilization by Indian mustard. Plant Physiol 117:1487-1494.
Esaki N, Nakamura T, Tanaka H, Suzuki T, Morino Y, Soda K (1981) Enzymatic synthesis of selenocysteine in rat liver. Biochemistry 20:4492-4496.
Franke KW (1934) A new toxicant occurring naturally in certain samples of plant foodstuffs. I. Results obtained in preliminary feeding trials. J Nutr 8:597-602.
Ganther HE (1979) Metabolism of hydrogen selenide and methylated selenides. Adv Nutr Res 2:107-128.
Ganther HE (1999) Selenium metabolism, selenoproteins and mechanisms of cancer prevention: Complexities with thioredoxin reductase. Carcinogenesis 20:1657-1666.
Gao X, Zhang J, Zhang L (2000) Acute toxicity and bioavailability of nano red elemental selenium (in Chinese). Wei Sheng Yan Jiu 29:57-58.
Garberg P, Stahl A, Warholm M, Hogberg J (1988) Studies on the role of DNA fragmentation in selenium toxicity. Biochem Pharmacol 37:3401-3406.
Goehring TB (1984) Toxic effects of selenium on growing swine fed corn-soybean meal diets. J Anim Sci 59:733-737.
Green DE, Albers PH (1997) Diagnostic criteria for selenium toxicosis in aquatic birds: Histologic lesions. J Wildlife Dis 33:385-404.
Hafeman DG, Sunde RA, Hoekstra WG (1974) Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr 104:580-587.
Halliwell B, Gutteridge JMC (2007) Free Radicals in Biology and Medicine. 4th ed. Oxford University Press, Oxford, 187-267.
Hasegawa T, Mihara M, Nakamuro K, Sayato Y (1996) Mechanism of selenium methylation and toxicity in mice treated with selenocystine. Arch Toxicol 71:31-38.
Hilton JW, Hodson PV, Slinger SJ (1982) Absorption, distribution, half-life and possible routes of elimination of dietary selenium in juvenile rainbow trout (Salmo gairdneri). Comp Biochem Physiol 71C: 49-55.
Ip C (1998) Lessons learned from basic research in selenium and cancer prevention. J Nutr 128:1845-1854.
Jariwalla RJ, Gangapurkar B, Nakamura D (2009) Differential sensitivity of various human tumour-derived cell types to apoptosis by organic derivatives of selenium. Br J Nutr 101:182-189.
Kelly MP, Power RF (1995) Fractionation and identification of the major selenium compounds in selenized yeast. J Dairy Sci 78 (Suppl 1):237.
Kelly SA, Havrilla CM, Brady TC, Abramo KH, Levin ED (1998) Oxidative stress in toxicology: Established mammalian and emerging piscine model systems. Environm Health Perspect 106:375-386
Kim YS, Jhon DY, Lee KY (2004) Involvement of ROS and JNK1 in selenite-induced apoptosis in Chang liver cells. Exp Mol Med 36:157-164.
Kryukov, GV, Castellano S, Novoselov SV, Loabnov AV, Zehtab O, Guigo R, Gladyshev VN (2003) Characterization of mammalian selenoproteins. Science 300:1439-1443.
Levander O (1986) Selenium. In Mertz W ed., Trace Elements in Human and Animal Nutrition. Academic Press, Orlando, pp. 209-280.
Low SC, Berry MJ (1996) Knowing when not to stop: Selenocysteine incorporation into eukaryotes. Trends Biochem Sci 21:203-208.
McDowell LR (1997) Minerals for grazing ruminants in tropical regions. Bull Univ Florida, pp. 1-69.
Mézes M, Matkovics B (1986) The molecular mechanism of lipid peroxidation and its measurement. In Csaba Gy ed., Actual Problems of Biology Vol 34 (In Hungarian) Medicina, Budapest, pp. 61-104.
Mézes M, Sályi G (1994) Effect of acute selenium toxicosis on the lipid peroxide status and the glutathione system of broiler chickens. Acta Vet Hung 42:459-463.
Moeasgaard S, Morrill R (2002) The need for speciation to realise the potential of selenium in disease prevention. In Ebdon L ed., Trace Element Speciation for Environment, Food and Health. Royal Society of Chemistry, London, 261-283.
Nebbia C, Gremmels JF, Soffietti MG (1990) Pathogenesis of sodium selenite and dimethylselenide acute toxicosis in swine: tissue and blood biochemical changes. Res Comm Chem Pathol Pharmacol 67:117-130.
Nilsonne G, Sun X, Nyström C, Rundlöf A-K, Fernandes AP, Björnstedt M, Dobra K (2006) Selenite induces apoptosis in sarcomatoid malignant mesothelioma cells through oxidative stress. Free Radic Biol Med 41:874-885.
Oremland RS, Switzer Blum J, Culbertson CW, Visscher PT, Miller LG, Dowdle P, Strohmaier FE (1994) Isolation, growth, and metabolism of an obligately anaerobic, selenate-respiring bacterium, strain SES-3. Appl Environ Microbiol 60:3011-3019.
Padmaja K, Somasekharaiah BV, Prasad ARK (1997) Inhibition of lipid peroxidation by selenium in chick embryos. Drug Chem Toxicol 20:79-98.
Pryzynska K (2002) Determination of selenium species in environmental samples. Microchim Acta 140:55-62.
Raisbeck MF (2000) Selenosis. Toxicology 16:465-479.
Rotruck JT, Pope AL, Ganther HA, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: Biochemical role as a component of glutathione peroxidase. Science 179:588-590.
Schrauzer GN (2003) The nutritional significance, metabolism and toxicology of selenomethionine. Adv Food Nutr 47:73-112.
Schrauzer GN, Ishmael D (1974) Effects of selenium and of arsenic on the genesis of spontanous mammary tumors in C3H mice. Ann Clin Lab Sci 4:441-447.
Schwarz K, Foltz CM (1957) Selenium as an integral part of factor 3 against dietary liver degeneration. J Am Chem Soc 79:3292-3293.
Scott ML, Nesheim MC, Young RJ (1982) Nutrition of the Chicken. 3rd ed. M. L. Scott & Associates, Ithaca, pp. 120-158.
Seko Y, Imura N (1997) Active oxygen generation as a possible mechanism of selenium toxicity. Biomed Environ Sci 10:333-339.
Seko Y, Saito Y, Kitahara J, Imura N (1989) Active oxygen generation by the reaction of selenite with reduced glutathione in vitro. In Wendel A ed., Selenium in Biology and Medicine. Springer, Berlin, pp. 70-73.
Shen HM, Yang CF, Liu J, Ong CN (2000) Dual role of glutathione in selenite-induced oxidative stress and apoptosis in human hepatoma cells. Free Radic Biol Med 28:1115-1124.
Smith GS, Watkinson JH (1984) Selenium toxicity in perennial ryegrass and white clover. New Phytologist 97:557-564.
Spallholz JE (1994) On the nature of selenium toxicity and carcinostatic activity. Free Radic Biol Med 17:45-64.
Spallholz JE (1997) Free radical generation by selenium compounds and their pro-oxidant toxicity. Biomed Environ Sci 10:260-270.
Spallholz JE 2001. Selenium and the prevention of cancer. Part II: Mechanisms for the carcinostatic activity of Se compounds. Bull Selenium – Tellurium Developm Assoc pp. 1-12.
Spallholz JE, Hoffman DJ (2002) Selenium toxicity: cause and effects in aquatic birds. Aquat Toxicol 57:27-37.
Sunde RA (1990) Molecular biology of selenoproteins. Annu Rev Nutr 10:451-474.
Surai PF (2006) Selenium in Nutrition and Health. Nottingham University Press, Nottingham, 109-150.
Terada A, Yoshida M, Seko Y, Kobayashi T, Yoshida K, Nakada M, Nakada K, Echizen H, Ogata H, Rikihisa T (1999) Active oxygen species generation and cellular damage by additives of parenteral preparations: Selenium and sulfhydryl compounds. Nutrition 15:651-655.
Terry N, Zayed AM, de Souza MP, Tarun AS (2000) Selenium in higher plants. Annu Rev Plant Physiol Plant Mol Biol 51:401-432.
US EPA (1987) Ambient water quality criteria for selenium – EPA-440/5-87-006. U.S. Environmental Protection Agency, Office of Water Regulation and Standards, Washington, pp. 1-23.
Wu L, McGarry L, Lanfear J, Harrison PR (1995) Altered selenium-binding protein levels associated with selenium resistance. Carcinogenesis 16:2819-2824.
Yan L, Spallholz JE (1993) Generation of reactive oxygen species from the reaction of selenium compounds with thiols and mammary tumor cells. Biochem Pharmacol 45:429-437.
Yang G, Wang S, Zhou R, Sun S (1983) Endemic selenium intoxication of humans in China. Am J Clin Nutr 37:872-875.
Zhang J, Wang H, Yan X, Zhang L (2005) Comparison of short-term toxicity between Nano-Se and selenite in mice. Life Sci 76:1099-1109.

Volume 53(Suppl.1): 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Effect of rosemary and garlic oil supplementation on glutathione redox system of broiler chickens

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ABSTRACT The purpose of present study was to investigate the effect of rosemary and garlic oils on the lipid peroxidation and glutathione redox system in the blood and liver of broiler chicken. Day-old Hubbard broiler chickens (n=200) were fed with commercial broiler feed (control) and supplemented with garlic oil (0.25 g kg^{-1}), rosemary oil (1.5 g kg^{-1}) or their combination (0.25 g kg^{-1} garlic oil and 1.5 g kg^{-1} rosemary oil). At the end of the growing period (42 days of age) blood and liver samples of 10 animals were taken from each group to determine malondialdehyde and reduced glutathione content and glutathione peroxidase activity. There were no significant differences in the blood plasma or in red blood cell haemolysates among the groups, but garlic oil supplementation increased significantly reduced glutathione content and both essential oils the glutathione peroxidase activity in liver. However, combination of the two oils caused increase of malondialdehyde content of liver together with significantly higher glutathione peroxidase activity as compared to the control. Due to the beneficial effect on glutathione redox system both essential oils – used solely – can be used to reduce the effects of oxidative processes in physiological conditions **Acta Biol Szeged 53(Suppl.1): (2009)**

KEY WORDS

garlic oil,
glutathione,
glutathione peroxidase,
malondialdehyde,
rosemary oil

Phytobiotics or essential oils have importance as natural antioxidants both in animal and human nutrition but their antioxidant capacity is measurable in comparison with synthetic antioxidants only at relatively high doses (Cuvelier et al. 1990) and it was proven mostly only in Fe^{2+}/ascorbate system (Bozin et al. 2006, 2007). The main biologically active components of rosemary oil are carnosol, carnosic acid and its esters (Boutekedjiret et al. 2003). Some previous experiments showed that phenolic compounds of rosemary oil, such as carnosic acid act as antioxidants such as α-tocopherol (McCarthy et al. 2001) and its antioxidant activity was higher than some synthetic antioxidants in vitro (Richheimer et al. 1996). It was also found that rosemary oil improve meat (McCarthy 2001; Smet et al. 2005; Govaris et al. 2007) and egg (Galobart et al. 2001) quality, improve the resistance of the polyunsaturated fatty acids and cholesterol against oxidative damage.

The main active component of garlic oil is alliin (S-alylcysteine sulfoxide) or its derivatives which are showed antioxidant capacity in vitro (Yamasaki et al. 1994). Among the different, lipid soluble organic sulphur components of garlic, such as diallyl-disulphide (DADS), S-ethyl-cysteine or n-acetyl-cysteine (NAC) also have antioxidant properties in vitro (Dwivedi et al. 1998) and significantly decreased the rate of lipid oxidation and oxymioglobin formation in minced beef which also proved their antioxidant properties (Yin and Cheng 2003).

Accepted June 19, 2009
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Materials and Methods

A total of 200 day-old Hubbard broiler chickens were divided randomly into four treatment groups. Control group was fed with commercial diet without added antioxidants, while the feed of the treatment groups supplemented with 1.5 g kg^{-1} rosemary oil, 0.25 g kg^{-1} garlic oil or combination of them (1.5 g kg^{-1} rosemary oil and 0.25 g kg^{-1} garlic oil), respectively. The feeding trial lasted for 42 days and at the end of growing period 10 animals from each group was exterminated and blood and liver samples were taken.

Blood samples were taken after cervical dislocation into EDTA–Na_2 containing tubes. Blood plasma and blood cells were separated by centrifugation (2500 g, 20 min). Red blood cells were haemolysed with 1:9 volume of redistilled water and by freezing (-20°C, 18 hours) and thawing (25 °C, 30 min). Liver samples were taken from the distal part of the right lobe and store at -18°C until analysis. Liver homogenates were made with 9-fold cold (4°C) physiological saline (0.65 % w/v NaCl). Determination of malondialdehyde content was carried out from the crude homogenate, while other parameters determined from the 10,000 g supernatant fraction of the homogenate.

Malondialdehyde content of blood plasma and red blood cells (RBC) haemolysate was determined according to Placer et al. (1966) as modified by Matkovics et al. (1988) while in liver homogenate according to Mihara et al. (1980). Reduced glutathione (GSH) content of blood plasma, RBC haemolysate and liver homogenate was measured as described by

Table 1. Malondialdehyde and reduced glutathione content and glutathione peroxidase activity of blood plasma of chickens fed with different essential oils (mean ±S.D.).

	Control	Garlic oil	Rosemary oil	Garlic and rosemary oil
MDA (μmol L^{-1})	4.41 ± 0.77	4.28 ± 0.53	4.54 ± 2.09	4.02 ± 0.97
GSH (mmol L^{-1})	6.61 ± 2.03	4.86 ± 1.19	5.52 ± 0.37	6.02 ± 0.89
GSHPx (U g^{-1} protein)	10.94 ± 2.94	8.24 ± 3.89	8.44 ± 0.47	9.23 ± 1.58

Table 2. Malondialdehyde and reduced glutathione content and glutathione peroxidase activity of RBC haemolysate of chickens fed with different essential oils (mean ±S.D.).

	Control	Garlic oil	Rosemary oil	Garlic and rosemary oil
MDA (μmol L^{-1})	9.23 ± 1.32	10.26 ± 1.01	10.26 ± 1.24	9.51 ± 1.88
GSH (mmol L^{-1})	10.82 ± 4.84	10.64 ± 2.56	9.32 ± 1.15	8.50 ± 3.18
GSHPx (U g^{-1} protein)	5.86 ± 1.65	6.87 ± 2.23	6.65 ± 0.96	6.10 ± 1.52

Sedlak and Lindsay (1968), while glutathione peroxidase (E.C. 1.11.1.9) according to Matkovics et al. (1988). Enzyme activity expressed to protein content which was determined by biuret method (Weichselbaum 1948) for blood plasma and RBC haemolysate or with Folin-phenol reagent for liver homogenate (Lowry et al. 1951).

Statistical evaluation of the data was performed using paired LSD test (Statistica™ 4.5, Statsoft Inc., 1993).

Results and Discussion

There were no significant differences in any of the measured parameters in blood plasma or RBC haemolysate (Tables 1 and 2). These results can be explained with the fast absorption, metabolic conversion and excretion of essential oil constituents that was mentioned by Kohlert et al. (2000). Additionally these results suggest that the effect of essential oils manifested mainly in tissues, but not in blood, possibly because of their lipid soluble characteristics. Lee et al. (2004) reported that in spite of the short half-life of the essential oils, those are able to accumulate in chicken tissues - mainly in liver and kidney - if the administration is continuous without withdrawal periods.

Malondialdehyde content of liver homogenates were not modified by the two essential oils if those were applied separately. However, combined treatment caused significantly higher malondialdehyde concentration (Table 3). That result can be explained by the findings of Skibola and Smith (2000) and also with those of Liu (2003), who found that excess amount of essential oils in the diet, may have not have anti-oxidant but pro-oxidant effect.

Glutathione content was significantly higher in liver homogenate as effect of garlic oil supplementation (Table 3). There are two explanation of that result. First, garlic oil contains organic sulphur compounds which may react with 5,5'-dithiobis-2 nitrobensoic acid used as colour complex reagent for the determination of reduced glutathione as non-protein sulphydryl group. Second, garlic oil prevents oxidation of glutathione through its active components, such as S-allylcysteine, that lead to functional recovery (Sener et al. 2007). Additionally garlic oil possibly improves the

Table 3. Malondialdehyde and reduced glutathione content and glutathione peroxidase activity of liver homogenates of chickens fed with different essential oils (mean ±S.D.).

	Control	Garlic oil	Rosemary oil	Garlic and rosemary oil
MDA (μmol g^{-1})	6.78 ±0.80^{a}	6.30 ± 1,23^{ab}	6.19 ± 1.05^{ab}	7.88 ± 1.14^{b}
GSH (mmol g^{-1})	0.85 ± 0.09^{a}	0.96 ± 0,11^{b}	0.91 ± 0.10^{ab}	0.97 ± 0.17^{ab}
GSHPx (U g^{-1} protein)	0.77 ± 0.09^{a}	0.90 ± 0,20^{b}	1.22 ± 0.14^{b}	1.18 ± 0.24^{b}

^{a,b} Different superscripts in the same row means significant difference at $P<0.05$ level

absorption of amino acids, such as methionine or cysteine, from the gastro-intestinal tract which are the limiting factors of glutathione synthesis (Wang et al. 1997).

Glutathione peroxidase activity was significantly higher in the 10,000 g supernatant fraction of liver homogenates of all three treated groups as compared to the control (Table 3). Glutathione peroxidase activity depends on the presence of its active centre, selenium (Sun et al. 1998), also its substrates, reduced glutathione (Németh et al. 2004) and oxygen free radicals which are first increase and at continuously high level decrease the enzyme activity (Holovska et al. 1996). The results suggest that garlic or rosemary oil as antioxidants decrease the oxygen free radical formation therefore increase the enzyme activity and the same effect was found if those were applied together, and based on the significantly higher malondialdehyde content, provoke free radical formation but only at low extent which increase the enzyme activity. Auroma et al. (1992) reported that carnosic acid appears to scavenge H_2O_2, but it could also act as a substrate for the peroxidase system, also there are some reports about the possible pro-oxidant effects of some garlic oil constituents (Amagase et al. 2001).

In conclusion the results showed that both garlic and rosemary oils have antioxidant properties and those have positive effect on the glutathione redox system of liver in chickens, but using together in a much higher amount those have opposite, pro-oxidant, effect.

References

Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y (2001) Intake of garlic and its bioactive components. J Nutr 131 (Suppl. 3):955S-962S.

Aruoma OI, Halliwell B, Aeschbach R, Löligers J (1992) Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. Xenobiotica 22:257-68.

Boutekedjiret C, Bentahar F, Belabbes R, Bessiere JM (2003) Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour Fragrance J 18:481-484.

Bozin B, Mimica-Dukic N, Simin N, Anackov G (2006) Characterization of the volatile com-position of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. J Agric Food Chem 54:1822-1828.

Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. (2007) Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. J Agric Food Chem 55:7879-7885.

Cuvelier ME, Berset C, Richard H (1990) Use of a new test for determining comparative antioxidant activity of butylated hydroxyanisoe, butylated hydroxytoluene, alpha- and gamma-tocopherols and extract from rosemary and sage. Sci Aliments 10:797-806.

Dwivedi C, Rohlfs S, Jarvis D, Engineer F (1992) Chemoprevention of chemically-induced skin tumor development by diallyl sulfide and diallyl disulfide. Pharm Res 9:1668-1670.

Galobart J, Barroeta AC, Baucells MD, Codony R, Ternes W (2001) Effect of dietary supplementation with rosemary extract and alpha-tocopheryl acetate on lipid oxidation in eggs enriched with omega 3-fatty acids. Poult Sci 80:460-467.

Govaris A, Florou-Paneri P (2007) The inhibitory potential of feed supplementation with rosemary and/or alpha-tocopheryl acetate on microbial growth and lipid oxidation of turkey breast during refrigerated storage. LWT Food Sci Technol 40:331-337.

C. Kohlert I, van Rensen R, März G, Schindler EU, Graefe M, Veit M (2000) Bioavailability and pharmacokinetics of natural volatile terpenes in animals and humans. Planta Med 66:495-505.

Lee KW, Everts H, Beynen AC (2004) Essential oils in broiler nutrition. Int J Poult Sci 3:738-752.

Liu RH (2003) Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 78: 517S-520S.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Matkovics B, Szabó L, Sz Varga I (1988) Determination of enzyme activities in lipid peroxidation and glutathione pathways (In Hungarian). Laboratóriumi Diagnosztika 15: 248-250.

McCarthy TL, Kerry JP, Kerry JF, Lynch PB, Buckley DJ (2001) Evaluation of the antioxidant potential of natural food / plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. Meat Sci 57:45-52.

Mihara M, Uchiyama M, Fukuzawa K (1980) Thiobarbituric acid value of fresh homo-genate of rat as parameter of lipid peroxidation in ageing, CCl_4 intoxication and vitamin E deficiency. Biochem Med 23:302-311.

Németh K, Mézes M, Gaál T, Bartos Á, Balogh K, Husvéth F (2004) Effect of supplementation with methionine and different fat sources on the glutathione redox system of growing chicken. Acta Vet Hung 52:369-378.

Placer ZA, Cushman LL, Johnson BC (1966) Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. Anal Biochem 16:359-364.

Richheimer SL, Bernart MW, King GA, Kent MC, Bailey DT (1996) Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. J Am Oil Chem Soc 73:507-514.

Sedlak I, Lindsay RH (1968) Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissues with Ellmann's reagent. Anal Biochem 25:192-205.

Sener G, Sakarcan A, Yegen BC. (2007) Role of garlic in the prevention of ischemia-reperfusion injury. Mol Nutr Food Res. 51:1345-1352.

Skibola CF, Smith MT (2000) Potential health impacts of excessive flavonoid intake. Free Radic Biol Med 29:375-383.

Smet K, Raes K, Huyghebaert G, Haak L, Arnouts S, De Smet S. (2005) Influence of feed enriched with natural antioxidants on the oxidative stability of broiler meat. Proc. 17th European Symposium on the Quality of Poultry Meat, Doorwerth, pp. 99-106.

Yamasaki T, Li L, Lau B (1994) Garlic compounds protect vascular endothelial cells from hydrogen peroxide-induced oxidant injury. Phytother Res 8:408-412.

Wang ST, Chen HW, Sheen LY, Lii CK (1997) Methionine and cysteine affect glutathione level, glutathione-related enzyme activities and the expression of glutathione S-transferase isozymes in rat hepatocytes. J Nutr 127:2135-2141.

Weichselbaum TE (1948) An accurate and rapid method for the determination of protein in small amounts of serum and plasma. Am J Clin Pathol 16:40-43.

Volume 53(Suppl.1): 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Effect of long term feeding of T-2 and HT-2 toxin contaminated diet on the glutathione redox status and lipid peroxidation processes in common carp (Cyprinus carpio L.)

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ABSTRACT Effect of sublethal T-2 (2.45 mg kg^{-1} feed) and HT-2 (0.52 mg kg^{-1} feed) toxin treatment for 4 weeks was investigated in common carp. Two groups, a control and a T-2+HT-2 toxin treated were formed. Six carps were exterminated from each group weekly. Liver samples were taken, in which reduced glutathione concentration and glutathione-peroxidase activity were measured. Free radical formation was measured by a direct reactive oxygen metabolites test, and also malondialdehyde concentration was determined. From the first week lower feed consumption and weight gain was recorded in T-2 toxin treated group, which resulted significantly lower live weight at the end of experiment. Feeding the T-2 toxin contaminated diet caused, significantly elevated glutathione concentration and glutathione-peroxidase activity during the first week. At second week the T-2 toxin loading resulted decrease in glutathione concentration and glutathione-peroxidase activity of liver as compared to the control. Although the reactive oxygen metabolite concentration was elevated in T-2 toxin treated group during the first two weeks, no significant changes were found in the MDA concentration. The results show that the biological antioxidant system was able to eliminate the harmful peroxidative effect of T-2 toxin in common carp.

Acta Biol Szeged 53(Suppl.1): (2009)

KEY WORDS

common carp
T-2 toxin
glutathioe redox system
lpid peroidailntroduction

Healthy environment (also healthy feed) is essential to maintain fish harvest level in the face of increasing demand. Moulds, and also their secondary metabolites, the mycotoxins are responsible for the impairment/mildew of feedstuffs either in field, or during transportation and/or storage (Lawlor and Lynch 2005). The occurrence of mycotoxins in cereal grains is a great concern, because their presence in feeds is often associated with chronic of acute mycotoxicoses in animals. Mycotoxins cause a wide variety of adverse effect (decrease of immune response, clinical signs) depending on the nature and concentration of mycotoxins present, the duration of exposure, the animal species, its age, health and nutritional status during the exposure to mycotoxin contaminated feed (Diaz 2005).

In fish feeds and feedstuffs several moulds are producing mycotoxins (e.g. Aspergillus, Penicillium and Fusarium). Moreover any mycotoxin could be produced from different mould species, and different moulds can produce the same mycotoxins (Jouany 2007).

With the environmental pressure on the aquaculture industry to reduce the level of fish meal in the diet with plant based proteins, the occurrence of mycotoxin contamination in fish feeds may increase. Although the presence the mycotoxin contaminated feeds in fish nutrition in tropical and subtropical areas of the world is relative high, few data are available about its toxic effect on fish species, their harmful effect on the biological antioxidant system and thereby the immune response and health status of the animals (Manning 2005).

In Hungary, the continental climate is preferable for Fusarium species, which produce a variety of thichotecene mycotoxins. Fusarium sporotrichoides is a widespread mould on plant and in the soils of the temperate climate of the world, producing 'type A' trichothecene mycotoxins, e.g. T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, scirpentriol, and diacetoxyscirpenol (Ueno 1983)..

The first report on toxicity of Fusarium mycotoxins in fish was published by Marasas et al. (1969). They found that low doses (0.2 or 0.4 mg kg^{-1}) of T-2 toxin derived from Fusarium tricinctum in the feed of rainbow trout (Salmo gairdneri) for 12 months failed to induce hepatoma and actually had a growth promoting effect. However, an acute dose (6.1 mg kg^{-1}) of T-2 toxin was more toxic to fingerlings than adults, although dose of 8 mg kg^{-1} severely damaged the intestinal tract of the fishes.

Accepted June 19, 2009
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Table 1. Measured nutrient content of diet.

Nutrient content (% dry matter)		
Crude protein		33.43 %
Crude fat		6.66 %
In which	saturated fatty acids	23.00 %
	monounsaturated fatty acids	35.70 %
	polyunsaturated fatty acids	40.90 %
Crude fiber		0.57 %
Nitrogen free extract		55.26 %
Ash		4.08 %

Woodward et al. (1983) found that deoxynivalenol (1 to 13 mg kg^{-1}) produced by Fusarium moulds in diet of rainbow trout led to progressively greater depression of weight gain ranged (from 12% to 92% of the control) and resulted from an adverse effect on both feed intake and feed conversion efficiency. Complete feed refusal occurred at 20 mg kg^{-1} deoxynivalenol. The T-2 toxin in different dietary concentration (0.625, 1.25, 2.5 and 5.0 mg kg^{-1} of diet) according to Manning et al. (2003) is responsible for significant reduction of growth (in all treatments), poor feed conversion (only at 5.0 mg kg^{-1} T-2 toxin level), adversely affected hematocrit value (at 1.25, 2.5 and 5.0 mg kg^{-1} T-2 toxin level), low survivability and histopathological anomalies of stomach and kidney in juvenile channel catfish.

There are few data about the effect of T-2 toxin on the xenobiotic transforming enzymes in fishes. Kravchenko et al. (1989) found that 0.46 mg kg^{-1} body weight T-2 toxin moderately increased glutathione-S-transferase (GST) activity in carp, whereas significantly increased (2-11 fold) activity of lysosomal enzymes and elevated (2-fold) alkaline phosphatase activity was also measured.

Materials and Methods

A total of 72 common carps (Cyprinus carpio L.) were obtained from a commercial fish farm and divided into 6 aquaria (150 L each), at a stocking density of 12 fish per aquaria. All aquaria were filled up with aerated dechlorinated tap water and were connected to a re-circulating system. Light regimen was maintained at a 12:12 h light:dark schedule. The aquaria were cleaned every day by syphoning out the debris and faeces. After 3 weeks of adaptation period, at the initial of the experiment two groups were formed, a control (3 aquaria) and a treated one (3 aquaria).

The diet of the control group (carp grower complete feed) did not contain detectable amounts of trichothence mycotoxins, while the feed of the treated group was contaminated by T-2 (2.45 mg kg^{-1}) and HT-2 (0.52 mg kg^{-1}) toxin. Mycotoxin analyses were carried out by HPLC method according to the standard method (Hungarian Feed Code 2004). The nutrient content of diet was determined according to the standard methods (Hungarian Feed Code 2004) and it is shown in Table 1.

T-2 and HT-2 toxin were produced experimentally on maize by Fusarium sporotrichioides strain NRRL 3299 (Agricultural Research Service Culture Collection, National Centre for Agricultural Utilization Research, Peoria, IL). Extraction and purification of toxin was carried out according to the method of Fodor et al. (2006).

The experiment lasted four weeks. At the 7th, 14th, 21st and 28th days of experiment 6-6 fish were sampled both from the control and the T-2 toxin treated group. They were weighed and exterminated. Liver samples were taken and were stored at -20°C (2 days), then at -70°C for further analysis.

The experiment was approved by the Animal Experimental Committee of the Faculty of Agricultural and Environmental Sciences of the Szent István University.

Biochemical analyses

For the biochemical analyses small amount (0.5 g) of the thawn liver samples were homogenized in nine-fold volume of isotonic saline (0.9% w/v NaCl).

Glutathione peroxidase (E.C.1.11.1.9) activity was measured in the 10,000 g supernatant fraction of liver homogenate, using reduced glutathione (GSH) and cumene hydroperoxide as co-substrates in an end-point direct assay following the system of Lawrence and Burk (1976). The loss of glutathione was measured using Ellmann's reagent (Sedlak and Lindsay 1968). The enzyme activity was expressed as nmol glutathione oxidation per minute at 25°C. The enzyme activity was calculated to protein content of the 10,000 g supernatant fraction of tissue homogenate, which was measured using Folin-phenol reagent (Lowry et al. 1951).

Table 2. Live weight of the sampled animals during the experiment.

	Live weight (g)			
	7th day	14th day	21st day	28th day
Control	53.45 ± 5.20	55.55 ± 8.53	56.60 ± 3.73	62.45b ± 6.48
T-2 and HT-2 toxin treated	52.35 ± 3.88	53.55 ± 6.26	54.52 ± 10.33	53.68a ± 5.61

All values are given as the mean ± SD; $n = 6$. Values in the same column with different superscripts significantly differ at $p < 0.05$.

Table 3. Effect of long-term feeding of T-2 and HT-2 toxin contaminated diet on the glutathione redox system of carps.

	GSH concentration (umol/g)			
	7th day	14th day	21st day	28th day
Control	1.68aA ± 0.20	1.74AB ± 0.28	2.15B ± 0.53	1.55aA ± 0.39
T-2 and HT-2 toxin treated	2.36bB ± 0.52	1.60A ± 0.21	2.44B ± 0.65	2.24bB ± 0.66
		GSHPx activity (U/g protein)		
	7th day	14th day	21st day	28th day
Control	1.87a ± 0.40	2.09 ± 0.37	2.36 ± 0.50	1.96 ± 0.51
T-2 and HT-2 toxin treated	2.60bAB ± 0.32	1.89A ± 0.42	2.84B ± 1.04	2.61AB ± 0.76

All values are given as the mean ± SD; $n = 6$. Values in the same column with different superscripts (a, b) significantly differ at $p < 0.05$. Values in the same row with different superscripts (A, B) significantly differ at $p < 0.05$.

GSH content of the 10,000 g supernatant fraction of liver homogenate was determined based on the colour complex formation of glutathione with Ellmann's reagent (Sedlak and Lindsay 1968).

Reactive oxygen metabolites (ROMs) of the 10,000 g supernatant fraction of liver homogenate were measured by a colorimetric determination kit (Diacron, Grosetto, Italy). In the test hydroperoxides in presence of iron are able to generate alkoxyl and peroxyl radicals. These radicals are able to oxidize an alkyl-substituted aromatic amine transforming them in a pink-colored derivative, which can be photometrically quantified (Cornelli et al. 1999).

Malondialdehyde (MDA) content of the liver homogenate (1:9 in physiological saline) was measured based on the colour complex formation of malondialdehyde with 2-thiobarbituric acid in an acidic environment at high temperature (Placer et al. 1966). The standard was 1,1,3,3-tetraethoxypropane (Fluka, Buchs, Switzerland).

Statistical analysis

After calculating the means and standard deviations (S.D.) statistical evaluation (paired t-tests, analysis of variance, linear regression analysis) of the results was carried out with Statistica™ for Windows 4.5 (Statsoft Inc., 1993) software.

Results

During the experiment no clinical signs of mycotoxicosis or mortality emerged. Live weight of fishes in the T-2 toxin treated group was significantly lower from the 1st week of the trial up to the end of experiment (Table 2).

Feeding the T-2 and HT-2 toxin contaminated diet increased the amount/activity of glutathione redox system during the 1st week, which is shown by the significantly elevated GSH concentration and glutathione peroxidase (GSHPx) activity of the 10,000 g supernatant fraction of liver homogenate (Table 3). At 2nd week of the trial the T-2 and HT-2 toxin treatment resulted decrease in GSH concentration and GSHPx activity of liver compared to control, but at the end of the experiment both parameters – GSH concentration even at $p<0.05$ level of significance – exceeded the values measured in control fishes (Table 3). In the T-2 and HT-2 toxin treated group the lowest GSH concentration and GSHPx activity was measured at 14th day of experiment which was significantly lower – in case of GSH concentration – than the values at the

Table 4. Effect of long-term feeding of T-2 and HT-2 toxin contaminated diet on the free radical generation and lipid peroxidation processes of carps.

	dROMs concentration (mg H_2O_2/dl)			
	7th day	14th day	21st day	28th day
Control	0.61 ± 0.34	0.81±0.46	2.05±0.36	1.52±1.21
T-2 and HT-2 toxin treated	1.52 ± 1.09	1.88±1.36	1.98±0.67	1.28±1.03
		MDA concentration (μmol/g)		
	7th day	14th day	21st day	28th day
Control	39.78BC ± 10.56	30.35B ± 2.86	16.29A ± 6.57	24.59AB ± 12.97
T-2 and HT-2 toxin treated	36.41C ± 5.47	34.70C ± 4.55	15.30A ± 1.94	24.54B ± 12.95

All values are given as the mean ± SD; $n = 6$. Values in the same column with different superscripts (a, b) significantly differ at $p < 0.05$. Values in the same row with different superscripts (A, B, C) significantly differ at $p < 0.05$.

Table 5. Summary of the linear regression analysis (linear regression coefficients) between the measured parameters of glutation redox system and lipid peroxidation processes in liver of carps. n.s. – non significant.

		GSHPx	MDA	dROMs
GSH control	r =	0.765	- 0.275	0.370
		p < 0.001	n.s.	n.s.
T-2 and HT-2 toxin treated	r =	0.913	- 0.019	- 0.087
		p < 0.001	n.s.	n.s.
GSHPx control	r =		- 0.258	0.191
			n.s.	n.s.
T-2 and HT-2 toxin treated	r =		- 0.106	- 0.063
			n.s.	n.s.
MDA control	r =			- 0.804
				P<0.001
T-2 and HT-2 toxin treated	r =			- 0.326
				n.s.

other samplings, and in case of GSHPx activity than at 21st day of experiment. (Table 3).

Although the reactive oxygen metabolite concentration was elevated in T-2 and HT-2 toxin treated group during the first 2 weeks of the trial, no significant changes were found in the MDA concentration between the T-2 and HT-2 toxin treated and the control carps (Table 4). Both in control and T-2 + HT-2 toxin treated groups significantly lower MDA concentration was measured at 21st day of experiment than at the other days of sampling (Table 4).

Linear regression analysis of the measured parameters showed close positive correlation between the GSH concentration and GSHPx activity of liver both in control ($r=0.765$; $p<0.001$) and T-2 and HT-2 toxin treated groups ($r=0.913$; $p<0.001$). Negative, although weak, correlation was found between the measured parameters of glutathione redox system (GSH concentration or GSHPx activity) and the MDA concentration in both experimental groups (Table 5). Negative linear correlation was found between the concentration of reactive oxygen metabolites (ROMs) and the MDA concentration in both experimental groups, which was strong in the control group ($r=-0.804$; $p<0.001$) (Table 5).

Discussion

The observed reduced live weight of the T-2 toxin treated group at the end of the 4 weeks long experiment are in line with the findings of Manning et al. (2003) in channel catfish, who used similar T-2 toxin concentrations to this experiment. Marasas et al. (1969) also observed this effect of T-2 toxin exposure in rainbow trout but in a long-term (12 month) feeding trial.

The changes of GSH concentration in liver of T-2 toxin treated group are slightly controversial to the findings of Kravchenko et al. (1989). In their study with common carp they found no effect of T-2 toxin (0.46 mg kg^{-1} body weight) on the GSH level of liver. Our result may show that T-2 toxin at the applied dose (3.0 mg kg^{-1}) at the beginning of exposure increases the amount (GSH) and activity (GSHPx) of glutathione redox system, which play important role in elimination of harmful peroxides. Later, at 14th day there were a small decrease both in the amount and activity of glutathione redox system in the T-2 toxin treated group compared to the values a week before, but increased again during the following weeks of the trial. These results suggest continuous activation of the glutathione redox system as effect against oxidative stress caused by T-2 and HT-2 toxin exposure.

Linear regression analysis showed close positive correlation between the GSH concentration and GSHPx activity of liver both in control and T-2 toxin treated groups, which are in line with our previous findings in broiler chickens (Balogh et al 2007). The co-substrate (GSH) surplus caused elevated GSHPx activity both at 7th and 28th day of experiment, but at 14th day it has resulted lower GSHPx activity than the control.

Although the reactive oxygen metabolite concentration was slightly elevated in T-2 toxin treated group during the first two weeks of T-2 and HT-2 toxin load, no significant changes were found in the MDA concentration, which shows that the biological antioxidant system was able to eliminatethe harmful peroxidative effec of T-2 toxin n commo capAcknowledgment

Acknowledgement

The research was supported by Hungarian Academy of Sciences Office for Research Groups Attached to Universities and Other Institutions (Project No. 2006TKI117). We wish to thank Mr. I. Dankó and Ms. K. Dankó for their generosity in providing the carps and Mr. B. Balogh for his technical assistance during the experiment.

References

Balogh K, Weber M, Erdélyi M, Mézes M (2007) Investigation of lipid peroxide and glutathione redox status of chicken concerning a high dietary selenium intake. Acta Biol Hung 59:269-279.

Cornelli U, Cornelli M, Terranova R (1999) Free-radical and vascular disease. *Intern Union Angiol Bull 15*:7-10.

Diaz D ed (2005) The Mycotoxin Blue Book. Nottingham University Press, Nottingham, pp. 349.

Fodor J, Kametler L, Kovács M (2006) Practical aspects of fumonisin production under laboratory conditions. Mycotox Res 22:211-216.

Hungarian Feed Code (2004) Determination of nutrients and undesirable substances (In Hungarian). Vol. II/II., OMMI-FVM, Budapest

Jouany JP (2007) Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. Anim. Feed. Sci. Technol. 137: 342-362.

Kravchenko LV, Galash VT, Avren'eva LT, Kranauskas AE (1989) On the sensitivity of carp, Cyprinus carpio, to mycotoxin T-2. J Ichthyol 29:156-160.

Lawlor PG, Lynch PB (2005) Mycotoxin management. Afr Farming Food

Process 46:12-13.

Lawrence RA, Burk RF (1976) Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Comm 71:952-958.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Manning B (2005) Mycotoxins in aquaculture. In Diaz D ed, The Mycotoxin Blue Book, Nottingham University Press, Nottingham, pp. 139-156.

Manning BB, Li MH, Robinson EH, Gaunt PS, Camus AC, Rottinghaus GE (2003) Response of channel catfish to diets containing T-2 toxin. J Aquat Anim Health 15:229-238.

Marasas WFO, Bamburg JR, Smalley EB, Strong FM, Ragland WL, Degurse BE (1969) Toxic effect on trout, rats and mice of T-2 toxin produced by the fungus Fusarium tricinctum (Cd.) Snyd. et Hans. Toxicol Appl Pharmacol 15:471-482.

Placer ZA, Cushman LL, Johnson BC (1966) Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem 16:359-364.

Sedlak I, Lindsay RH (1968) Estimation of total, protein-bound and non-protein sulfhydryl groups in tissues with Ellmann's reagent. Anal Biochem 25:192-205.

Ueno Y (1983) Trichothecenes: Chemical, Biological and Toxicological Aspects. Developments in Food Science. Elsevier, New York, pp. 1-60.

Woodward B, Young LG, Lun AK (1983) Vomitoxin in diets of rainbow trout (Salmo gairdneri). Aquaculture 35:93-101.

Volume 53(Suppl.1): 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Isoform specific expression of Δ9 desaturases in two brain regions of common carp

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ABSTRACT An appropriate ratio of saturated and unsaturated fatty acids contributes to membrane fluidity. The Δ9 stearoyl-CoA desaturases are key enzymes involved in the regulation of the lipid composition of cellular membranes. The expressions of the Δ9 stearoyl-CoA desaturase genes are regulated by many environmental factors, such as temperature changes and metal exposure, mostly at the levels of transcription and mRNA stability. The present study analyzes the effects of cold shock and Cd^{2+} treatment on the regulation of two Δ9 desaturase genes in the olfactory lobe and the cerebellum of common carp. The effect of Cd^{2+} was also followed on the expressions of the Δ9 desaturase genes of cold-shocked animals. Sudden cold shock or Cd^{2+} exposure induced Δ9 desaturase expressions in a time-, brain region- and isoform-specific manner. In contrast, both Δ9 desaturase genes were repressed by Cd^{2+} in cold-shocked animals.
Acta Biol Szeged 53(Suppl.1): (2009)

KEY WORDS

brain
carp
cold
shock
Δ9
desaturase
heavy metal

Diverse physiological and environmental stresses (*e.g.* hyper- and hypothermia, oxidative injury and heavy metals) produce multiple intracellular changes. Organisms respond to environmental challenges by adaptive responses. Maintenance of the appropriate membrane fluidity is an important adaptive process during temperature shock. The ability of cells to modulate the physical characteristics of the membrane lipids is mainly determined by the action of desaturases. Desaturases are enzymes essential for introducing double bonds into fatty acids (Lee et al. 1990; Nakamura et al. 2002). Δ9 stearoyl-CoA desaturase (Scd, Cds, Δ9 desaturase) is responsible for the introduction of the first *cis* double bond at position Δ9. *Scd* cDNA has been isolated from a variety of vertebrate species. Three variants of the *Scd* gene have been found in rodents (Thiede et al. 1986; Ntambi et al. 1988; Kaestner et al. 1989; Ideta et al. 1995). A single Δ9 desaturase gene has been identified from grass carp (*Ctenopharyngodon idella*), milkfish (*Chanos chanos*) (Chang et al. 2001; Hsieh et al. 2001; Hsieh et al. 2005), thilapia (*Oreochromis mossambicus*) (Hsieh et al. 2004) and zebrafish (*Danio rerio*) (Hsieh et al. 2003). In common carp, (*Cyprinus carpio*) two Δ9 desaturase (*Cds*) genes (*Cds1* and *Cds2*) have been reported (Tiku et al. 1996; Trueman et al. 2000; Polley et al. 2003).

The tissue distribution of the Δ9 desaturases has been extensively studied in a number of fish species. In milkfish, Δ9 desaturase was detected in the liver, brain, kidney, gill, heart and muscle (Hsieh et al. 2001), whereas the Δ9 desaturase transcript is highly expressed in the liver of grass carp, common carp, tilapia and zebrafish, but is not or only barely detectable in the brain (Hsieh et al. 2003; Polley et al. 2003).

To maintain homeostasis under cold shock, ectothermal teleosts increase the membrane fluidity through an increase in the proportion of unsaturated fatty acids in the cell membranes (Cossins et al. 2002; Valko et al. 2005). In common carp, it has been demonstrated that progressive cooling causes a large increase in Δ9 desaturase expression in the liver and the *Cds2* gene is transiently upregulated a few days after cold treatment (Tiku et al. 1996). It has also been shown that the carp liver possesses a latent, inactive form of Δ9 desaturase proteins that is activated shortly after the onset of progressive cooling, before the synthesis of the new Δ9 desaturase (Trueman et al. 2000).

Membrane phospholipids of aerobic organisms are continually subjected to oxidant challenges from endogenous and exogenous sources. Toxic chemical pollutants (especially heavy metals) are important sources of reactive oxygen species in biological systems. It is possible that metal ions cause changes in membrane fluidity; stimulating lipid peroxidation by oxidizing poly-unsaturated fatty acids and causing other damage (Kudo and Waku 1996; Cavaletto et al. 2002). Cadmium itself is unable to generate free radicals directly, but its indirect generation of superoxide and hydroxyl radicals and nitric oxide has been reported (Waisberg et al. 2003; Valko et al. 2005).

It has been observed that the Δ9 desaturase activity in rat hepatocytes is suppressed by Cd^{2+} exposure (Kudo et al. 1991). However, Cd^{2+} itself does not inhibit microsomal Δ9 desaturase activity *in vitro*. It has been suggested that the reduction of the Δ9 desaturase is due to a secondary effect,

Accepted June 19, 2009
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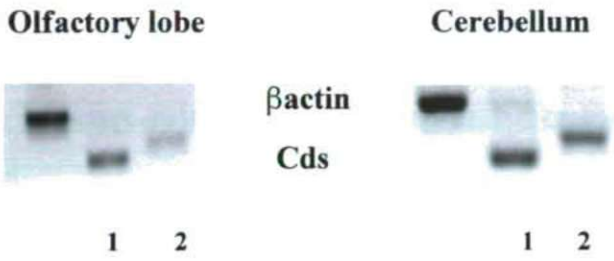

Figure 1. Expressions of carp *Cds1* and *Cds2* genes in the olfactory lobe and cerebellum of untreated animals. A representative result of the RT-PCR amplification. For *Cds1* primer pair Cds11F-CdsR was used, while primers Cds2F/CdsR amplified the *Cds2*-specific sequences. In parallel with the *Cds* isoform-specific transcripts, β-actin mRNA was amplified and used as an internal control to determine the relative levels of the two *Cds* transcripts.

brought about through changes in other factors, such as the insulin or glucose level.

One aim of the present study was to learn the consequences of a sudden temperature drop on the expressions of the two the Δ9 desaturase genes in the brain. We also investigated the regulation of the Δ9 desaturase genes of common carp exposed to Cd^{2+} under physiological and cold-shocked conditions, in brain regions with different levels of protection by the blood-brain barrier.

Materials and Methods

Animals and treatments

Carp were acclimatized under fasting conditions over a 3-week period at 12°C. In cold shock experiments fish were transferred from 12°C to 5°C for 1 and 5 h and samples were taken from the tissues immediately after the cold treatment. For Cd exposure the carp were transferred into 100 l water tanks containing 10 mg/l Cd^{2+} ($Cd(CH_3COO)_2x2H_2O$, Sigma-Aldrich Germany, Steinheim) for up to 126 h under static conditions. In the combined experiment, the fish were first kept at 5°C for 1 or 5 h and subsequently exposed to Cd^{2+} at 10 mg/l immediately after the cold shock. In all experiments, 4 animals were sacrificed for organ harvesting at each time point.

RNA extraction, reverse transcription and PCR amplification

Brain was homogenized in RNAzol B reagent (Tel-Test, Inc. USA, Texas) and total RNA was prepared according to the procedure suggested by the manufacturer. Total RNA was routinely treated with 100 U RNAse-free DNAseI (Fermentas, Lithuana, Vilnius) to avoid any DNA contamination.

To detect carp *Cds* specific mRNAs, an RT-PCR-based strategy was employed as earlier described (Hermesz et al. 2001).

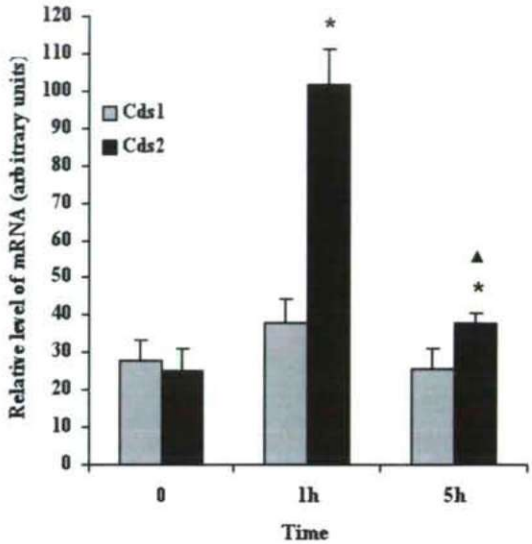

Figure 2. Time courses of expressions of the *Cds1* and *Cds2* genes in the olfactory lobe, followed by hypothermia. 1h and 5h indicate the duration of cold treatments. All data are means ± S.D. of the results of measurements on 4 animals at each time point. Significant difference: *, between the control (0) and the value at a given time point; ▲, between the values at consecutive time points of treatments.

Measurement of *Cds* mRNA levels

For amplification of carp *Cds* mRNAs, primers Cds11F, Cds2F and CdsR were used. The sequences of the primers were derived from the common carp *Cds* sequences (GenBank Accession Nos.: AJ249259 and U31864). Primers Cds11F and Cds2 are specific to *Cds1* and *Cds2*, respectively, and were used in pairs with CdsR recognizing both *Cds1* and *Cds2* sequences. The sequences of primers β-actin3 and 4 were derived from GenBank entry M24113 and used to amplify β-actin mRNA for internal standard.

Primers: Cds11F: 5'ccgcgtgcgctacattcgct3', Cds2F: 5'ttcttgtgtttctcagatca3', CdsR: 5'acgagtccatacagagctccg3', β-actin3: 5'gcaagagaggtatcctgacc3', β-actin4: 5'ccctcgtagatgggcacagt3'.

Images of ethidium bromide-stained agarose gels were digitized with a GDS 7500 Gel Documentation System and analyzed with GelBase/GelBlot™ Pro Gel Analysis Software (UVP). The relative levels of *Cds* mRNAs are expressed as ratios [*Cds*/β-actin x100].

Statistical analysis

For each time point of the experiments, 4 fish were used. RT-PCR reactions for each sample were performed in triplicate to increase the reliability of the measurements. Statistical differences were calculated with one-way analysis of variance (ANOVA) (MedCalc Statistical Software version 9.4.2.0,

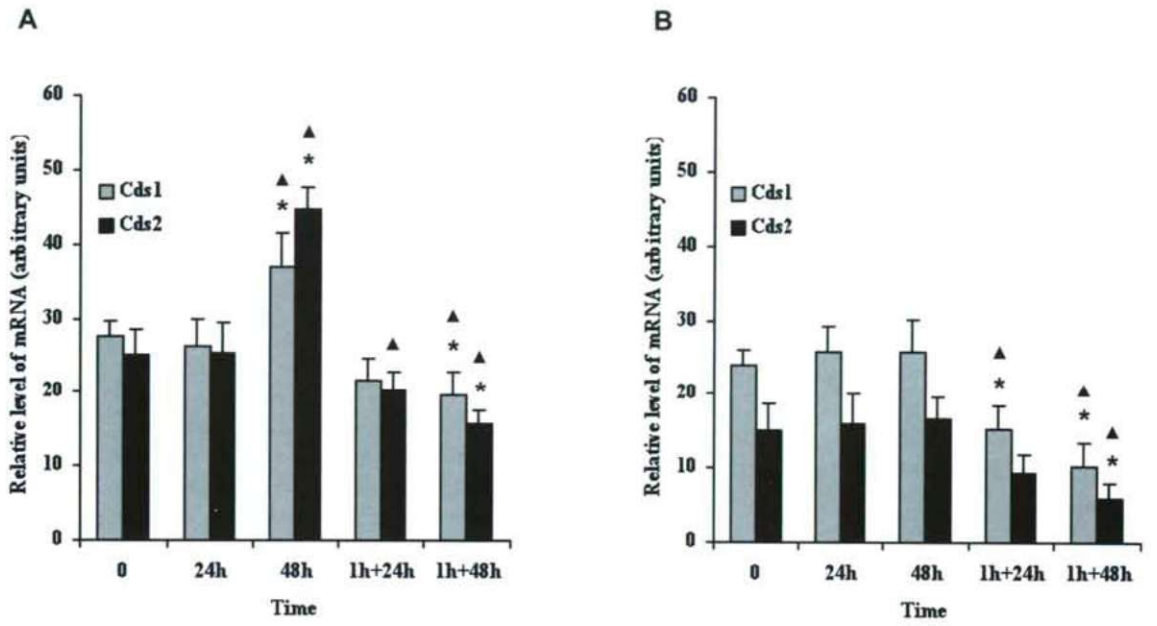

Figure 3. Effects of 10 mg/l Cd^{2+} on the expressions of the *Cds1* and *Cds2* genes in the (A) olfactory lobe and (B) in the cerebellum. 24h and 48h indicate the time period of Cd^{2+} treatment. 1h+24 and 1h+48 indicate the duration of cold shock followed by a 24h and 48h of Cd^{2+} treatments at the acclimatization temperature. All data are means ± S.D. of the results of measurements on 4 animals at each time point. Significant difference: *, between the control (0) and the value at a given time point; ▲, between the values at consecutive time points of treatments.

Broekstraat, Belgium) with a Student-Newman-Keuls follow-up test. Significant difference was accepted at $P<0.05$.

Results

Basal level of expression

For the detection and semiquantitative determination of *Cds1* and *Cds2* transcripts, isoform-specific primers were designed and used in RT-PCR reactions. In the control animals, both *Cds1*- and *Cds2*-specific mRNAs were readily detected in the two brain regions examined. The amount of *Cds2* mRNA was always less than that of *Cds1* mRNA in the olfactory lobe, with a ratio *Cds1*/*Cds2* of ~2:1, while in the cerebellum a modest excess of *Cds1* was detected; *Cds1*/*Cds2* ~1.2-1 (Fig. 1).

Effect of hypothermia

The *Cds1* and *Cds2* expressions demonstrated time-dependent and brain region-specific induction when the carp were exposed to a sudden 7°C decrease in temperature.

In the olfactory lobe, *Cds2* was transiently induced. A 4-fold increase in the level of *Cds2* mRNA was detected after 1 h of cold treatment. This induced level had dropped to 150% of the control value by 5 h of cold shock. The *Cds1* gene was less inducible; after 1 h of cold treatment, a modest (but not significant) 1.5-fold increase in mRNA level was measured (Fig. 2). In the cerebellum, only the *Cds2* gene was induced by the temperature drop; the mRNA level was increased ~1.5-fold after the 1-h cold exposure, but this change was not significant (data not shown).

Effect of Cd

Cd^{2+} at 10 mg/l proved to be an inefficient inducer of the *Cds* genes in the two brain regions examined. In the olfactory lobe, there were at most 1.5- and 2-fold increases in the levels of the *Cds1* and *Cds2* transcripts, respectively, with a maximum at 48-96 h. By 120 h of treatment, the expressions of both *Cds* genes had returned to the control level (data not shown and Fig. 3A). In the cerebellum, no significant changes in *Cds* transcript level were measured during the 120-h exposure (data not shown and Fig. 3B). A striking difference was found in the expressions of the *Cds* genes in the combined experiment when cold-shocked carp were exposed to Cd^{2+} at 10 mg/l for 24 and 48 h at 12°C, immediately after 1 h or 5 h of cold shock; the expressions of both *Cds* genes were suppressed. After 24 h of Cd^{2+} challenge, 20% and 10% decreases in the *Cds1* and *Cds2* mRNA levels were detected, respectively, in the olfactory lobe of cold-shocked carp. Cd^{2+} exposure for 48 h caused a further reduction (25-30%) in both mRNA levels (Fig. 3A). In the cerebellum, the downregulation of the *Cds* genes was more prominent after the combined exposure; 40% and 60% decreases in both mRNA levels were detected after 24 h and 48 h of Cd^{2+} exposure of cold-shocked animals, respectively (Fig. 3B). A 24-h or 48-h Cd^{2+} challenge following

5 h of cold exposure resulted in very similar suppression rates of both *Cds* genes; the data did not differ significantly from those measured after 1 h of cold shock.

Discussion

We report here the transcriptional regulation of two *Cds* genes in two brain regions of carp exposed to Cd^{2+} under physiological and cold-shocked conditions. We also show that both *Cds1*- and *Cds2*-specific mRNAs are readily detectable in the brain of the common carp and the distribution of the mRNAs exhibits isoform- and brain region-specificity. The tissue distribution of the Δ9 desaturases has previously been extensively studied in many fish species, and in most cases their expressions proved to be barely or not detectable at all in the brain (Hsieh et al. 2003; Polley et al. 2003). Differences in the expression profiles of the Δ9 desaturase genes between vertebrates possibly reflect species-specific differences in the requirements of these proteins in physiological adaptation (Chang et al. 2001).

No data have been published on the expressions of the Δ9 desaturase genes under stress conditions in the fish brain. We now report the first evidence that sudden cold shock induces the expressions of the *Cds* genes in the brain of the common carp. This expression is regulated spatially and temporally in an isoform-specific manner. The effect of cold shock on the *Cds* expression was investigated earlier in the carp liver (Schünke et al. 1983; Tiku et al. 1996; Polley et al. 2003). The hepatic Δ9 desaturase transcript was transiently upregulated a few days after slow progressive cooling treatment from 30 °C to 23, 17 and 10 °C. While modest cooling (to 23°C) during 1 day had no effect on the *Cds* expression, the most severe temperature drop (to 10°C) led to a large increase in *Cds* mRNA level. We found that the expression of *Cds2*, but not *Cds1*, was significantly affected by cold shock. In the liver, the two Cds genes were differentially regulated; the Cds2 gene was transiently upregulated by progressive cooling. The peak expression was measured during the cooling procedure to 10°C, but after exposure to 10°C for a day the level of the *Cds2* transcript was decreased dramatically. The control of desaturase expression seems to be very complex and may be related to the extent of cooling (Trueman et al. 2000).

The effect of Cd^{2+} loading on the *Cds* expression has previously been investigated only in the liver and in cultured rat hepatocytes (Kudo et al. 1990; Kudo et al. 1991). It has been shown that exposure to Cd^{2+} causes changes in the fatty acid composition of the phospholipids; the extent of formation of the 18:1 unsaturated fatty acid is reduced, while that of the 20:4 acid is slightly increased, as a result of the suppression of Δ9 and the induction of Δ6 desaturases (Kudo and Waku 1996). No data are available concerning the expressions of the *Cds* genes following metal exposure in fish. The present study has reveled that Cd^{2+} exposure induces the expressions of the *Cds* genes in the brain of the common carp. The two brain regions exhibited characteristic differences in sensitivity to Cd^{2+} and the effects of metal treatment were time-dependent. While the cerebellum proved to be unaffected by Cd^{2+}, in the olfactory lobe significant increases were measured in the expressions of both *Cds* genes. The olfactory lobe is not perfectly protected by the blood-brain barrier (Wong and Klaass 1982), and therefore its possible Cd^{2+} content might be an explanation for the elevated *Cds* mRNA level. This result contrasts with the effects of Cd^{2+} on the *Cds* activities in the rat liver and cultured hepatocytes (Kudo et al. 1990), where the Δ9 desaturase activities are reduced due to the direct action of Cd^{2+} on the liver.

We also followed the effects of Cd^{2+} on the *Cds* regulation in the brain of cold-shocked animals. In this combined treatment, the expressions of both *Cds* genes were suppressed in an isoform- and brain region-specific manner. It is interesting that the level of suppression correlated with the extent of Cd^{2+} exposure, whereas it was independent of the period of cold treatment. It has previously been shown that in the liver of Cd^{2+}-challenged rat the changes in Δ9 desaturase activity are greater in Zn^{2+}-deficient animals (Kudo et al. 1990). The cerebellum displays an altered *Cds* expression similar to that measured in the olfactory lobe. Since the cerebellum is protected by the blood-brain barrier, an indirect effect must be involved in the regulation of the *Cds* expression of cold-shocked animals. Measurement of the Zn^{2+} concentration in different tissues of cold-shocked animals revealed that the Zn^{2+} content in the brain was dramatically decreased (~ 50%) after 1 and 5 h of cold exposure (Hermesz, unpublished). The Δ9 desaturase expression is known to be regulated at the transcriptional level in response to various biological factors, such as the levels of essential fatty acids, insulin and glucose in the blood (Waters and Ntambi 1994). Since the Zn^{2+} concentration influences the insulin and glucose levels in the blood *in vivo*, we suggest that the reduction in the level of *Cds* mRNA is due to a secondary effect of Cd^{2+} exposure through changes in Zn^{2+} content and other factors, such as the insulin or glucose level.

In conclusion: The two Δ9 desaturase genes examined in different brain regions in this work exhibited unique basal expressions and characteristic sensitivities to Cd^{2+} treatment and a temperature drop. A sudden short cold shock influences the *Cds2* expression similarly as measured during progressive cooling. Cd^{2+} exposure of cold-shocked animals suppresses the *Cds* expression in a brain region-independent manner.

References

Chang BE, Hsieh SL, Kuo CM (2001) Molecular cloning of full-length DNA encoding delta-9 desaturase through PCR strategies and its genomic organization and expression in grass carp (*Ctenopharyngodon idella*). Mol Rep Devel 58:245-254.

Cavaletto M, Ghezzi A, Burlando B, Evangelisti V, Ceratto N, Viarengo A (2002) Effect of hydrogen peroxide on antioxidant enzymes and metallothionein level in the digestive gland of *Mytilus galloprovincialis*. Comp Biochem Physiol C 131:447-455.

Cossins AR, Murray PA, Gracey AY, Logue J, Polley S, Caddick M, Brooks S, Postle T, Maclean N (2002) The role of desaturases in cold-induced lipid restructuring. Biochem Soc Trans 30:1082-1086.

Hermesz E, Ábrahám M, Nemcsók J (2001) Tissue-specific expression of two metallothionein genes in common carp during cadmium exposure and temperature shock. Comp Biochem Physiol C 128:457-456.

Hsieh SL, Liao WL, Kuo CM (2001) Molecular cloning and sequence analysis of stearoyl-CoA desaturase from milkfish (*Chanos chanos*). Comp Biochem Physiol B 130:467-477.

Hsieh SL, Liu RW, Wu CH, Kuo CM (2003) Nucleotide sequence of a cDNA clone coding for stearoyl-CoA desaturase and its embryo expression in zebrafish (*Danio rerio*). Mol Rep Dev 66:325-333.

Hsieh SL, Chang HT, Wu CH, Kuo CM (2004) Cloning, tissue distribution and hormonal regulation of stearoyl-CoA desaturase in tilapia, *Oreochromis mossambicus*. Aquaculture 230:527-546.

Hsieh SL, Kuo CM (2005) Stearoyl-CoA desaturase expression and fatty acid composition in milkfish (*Chanos chanos*) and grass carp (*Ctenopharyngodon idella*) during cold acclimation. Comp Biochem Phys B 141:95-101.

Ideta R, Seki T, Adachi K (1995) Sequence analysis and characterization of FAR-17c, an androgen-dependent gene in the flank organs of hamsters. J Dermatol Sci 9:94-102.

Kaestner KH, Ntambi JM, Kelly TJ, Lane MD (1989) Differentiation-induced gene expression in 3T3-L1 preadipocytes. A second differentially expressed gene encoding stearoyl-CoA desaturase. J Biol Chem 264:14755-14761.

Kudo N, Nakagawa Y, Waku K (1990) The effect of cadmium on the composition and metabolism of hepatic fatty acids in zinc-adequate and zinc-deficient rats. Toxicol Lett 50(2-3):203-212.

Kudo N, Nakagawa Y, Waku K, Kawashima Y, Kozuka H (1991) Prevention by zinc of cadmium inhibitionof stearoyl-CoA desaturase in rat liver. Toxicology 68:133-142.

Kudo N, Waku K (1996) Cadmium suppresses Δ9 desaturase activity in rat hepatocytes. Toxicology 114:101-111.

Lee JA, Cossins AR (1990) Temperature adaptation of biological membranes: differential homoeoviscous responses in brush-border and basolateral membranes of carp intestinal mucosa. Biochim Biophys Acta 1026(2):195-203.

Macartney AI, Tiku PE, Cossins AR (1996) An isothermal induction of delta 9-desaturase in cultured carp hepatocytes. Biochim Biophys Acta 1302(3):207-216.

Nakamura MT, Nara TY (2002) Gene regulation of mammalian desaturases. Biochem Society Transact 30:1082-1086.

Ntambi JM, Buhrow SA, Kaestner KH, Christy RJ, Sibley E, Kelly TJ, Lane MD (1988) Differentiation-induced gene expression in 3T3-L1 preadipocytes. Characterization of a differentially expressed gene encoding stearoyl-CoA desaturase. J Biol Chem 263:17291-17300.

Polley SD, Tiku PE, Trueman RT, Caddick MX, Morozov IY, Cossins AR (2003) Differential expression of cold- and diet-specific genes encoding two carp liver delta 9-acyl-CoA desaturase isoforms. Am J Physiol Regul Integr Comp Physiol 284:41-50.

Schünke M, Wodtke E (1983) Cold-induced increase of and desaturase activities in endoplasmic membranes of carp liver. Biochim Biophys Acta 734:70-75.

Thiede MA, Ozols J, Strittmatter P (1986) Construction and sequence of cDNA for rat liver stearoyl coenzyme A desaturase. J Biol Chem 261:13230-13235.

Tiku PE, Gracey AY, Macartney AI, Beynon RJ, Cossins AR (1996) Cold-induced expression of delta 9-desaturase in carp by transcriptional and posttranslational mechanisms. Science 271:815-818.

Trueman RJ, Tiku PE, Caddick MX, Cossins AR (2000) Thermal thresholds of lipid restructuring and delta 9-desaturase expression in the liver of carp (*Cyprinus carpio* L.). J Exp Biol 203:641-650.

Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12(10):1161-1208.

Waisberg M, Joseph P, Hale B, Beyersmann D (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192:95-117.

Waters KM, Ntambi JM (1994) Insulin and dietary fructose induce stearoyl-CoA desaturase 1 gene expression of diabetic mice. Biol Chem 269(44):27773-27777.

Wodtke E, Cossins AR (1991) Rapid cold-induced changes of membrane order and activity in endoplasmic reticulum of carp liver: a time-course study of thermal acclimation. Biochim Biophys Acta 1064:343-350.

Wong KL, Klaassen CD (1982) Neurotoxic effects of cadmium in young rats. Toxicol Appl Pharmacol 63(3):330-337.

Volume 53(Suppl.1): 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Mineral elements in muscat sage plant (*Salvia sclarea* L.) and essential oil

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ABSTRACT The authors investigated the element content of different parts of muscat sage plant (*Salvia sclarea* L.) by ICP-OES for 18 elements (Al, B, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, V, Zn) and the composition of muscat sage oil during distillation by GC-MS. The essential oil was obtained by steam distillation. High Li and Cr concentration was found in plant samples. The essential oil was characterized by four main components: linalool, carvon, linalyl acetate and geranyl acetate and the composition of oil was unchanged during distillation.
Acta Biol Szeged 53(Suppl.1): (2009)

KEY WORDS

Salvia sclarea L.
metal ion content
GC-MS
essential oil

Medicinal pants take a prominent part in phytotherapy for treatment of illnesses. Some herbs, as muscat sage (*Salvia sclarea* L.) generally used in cosmetic industry as well (Szentmihályi et al. 2001). It is most frequently applied in aromatization of candles for deodorization and against depression, stress. Its effect is affirmed with juniper, lemon, geranium, jasmine and spike oil.

Salvia sclarea is a cultivated, biennial plant in Central Europe. Sporadically it flowers in the first year, although usually only in the second year in June-July and later in September. The linalyl acetate content characterizes the fregrance of muscat sage oil which is the highest one during the first flowering period in the second year. The ester value, calculated as linalyl acetate and used for the standardization of the oil may be as high as 70%. Ester values of French and English oils are 54-70% and 34-53%, respectively. The free linalool, l-8-cineole and limonene contents are also characteristic compounds with their highest level in the second year as well.

The essential oil composition in leaves differs from that of in flowers. Main constituents of leaf oil are α-thujone, l.8-cineole and terpenic acid and bornyl acetate is a characteristic ester in it. At the same time the essential oil composition depends on the environment where the plant grown up and the extraction method as well (Bernáth et al. 1991). Supercritical fluid extraction gives essential oil of different quality (Illés et al. 1994; Simándi et al 1996).

Element content in flowering plant of muscat sage, aqueous and some alcoholic extracts determined earlier (Szentmihályi et al. 2004).

Accepted June 19, 2009
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The object of our investigations was to study the element content of different parts of muscat sage by ICP-OES and the characteristics of muscat sage oil by GC-MS.

Materials and Methods

The plant material (muscat sage, *Salvia sclarea* L. [8163]) originates from the Botanical and Economical Research Institute of the Hungarian Academy of Sciences, Vácrátót. The examined parts of muscat sages were as follows: leaf, stem, calyx-leaf, bracteol, petal and flowering shoot.

Essential oil was obtained by steam distillation by description of the Hungarian Pharmacopoeia (Ph.Hg.VIII).

The determination of polyphenol content and oil yield was measured by the description of Szőke and Kéry 2003).

Microscopical evaluation was occured with scanning microscope (Hitachi 264 ON).

Thin layer chromatogram of essential oil components was done according to Wagner and Blandt (1996). Toulene-ethyl acetate (95:5) was used for development of plate and the spray reagent was anillin-sulfuric acid.

GC-MS was performed with a coupled system Agilent 6890N GC, 5973N mass selective detector, the Chrom Card Server Ver. 1.2. equipped with A HP-5MS capillary column, 30 m long, 0.25 mm id., 0.25 μm film thickness was used. Carrier gas was Helium (pHe was 0.20 MPa), at 1ml/min flow rate: 1 μL (10 μL/mL essential oil in ethanol) was injected at 0.7 mg/ml velocity, splitless-type with an Agilent 7683 autosampler. Temperature of injector was 280°C, temperature of transfer line was 275°C. Oven temperature was programmed initially at 60°C for 3 min, then increased with a rate of 8°C/min to 200°C, then kept at 200°C for 2 min and also increased with a rate of 10°C/min to 250°C with a final isotherm at

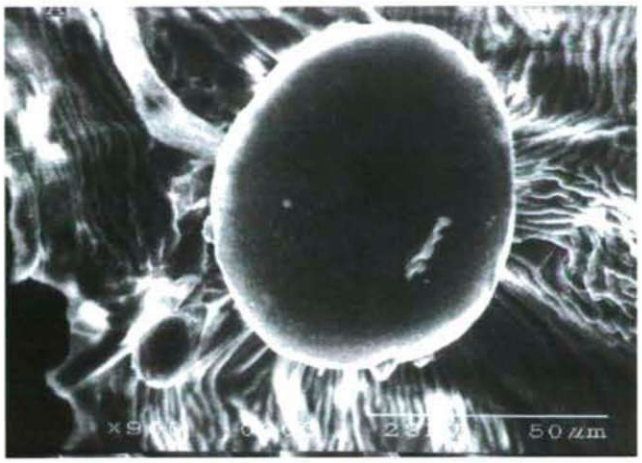

Figure 1. Surface of a grandular hair by scanning microscopy.

250°C for 15 min. MS conditions: ionization energy was 70 eV, mass range was 40-500 m/z, 1 analysis / min was made. Identification of peaks was carried out by comparison with MS and retention data of standards, and spectra from the NIST library.

The element concentration of samples was determined by ICP-AES (inductively coupled plasma optical emission spectrmetry) by method of Then et al. (2003) Type of instrument: Atom Scan 25 (Thermo Jarrell Ash), a sequential plasma emission spectrometer. Sampling: The dry milled samples (0.5 g) were digested with a mixture of HNO_3 (5 cm^3) and H_2O_2 (3 cm^3) in teflon vessels After digestion the samples were diluted to 25 cm^3, from which the following 18 elements were determined in three parallel measurements: Al, B, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, V, Zn.

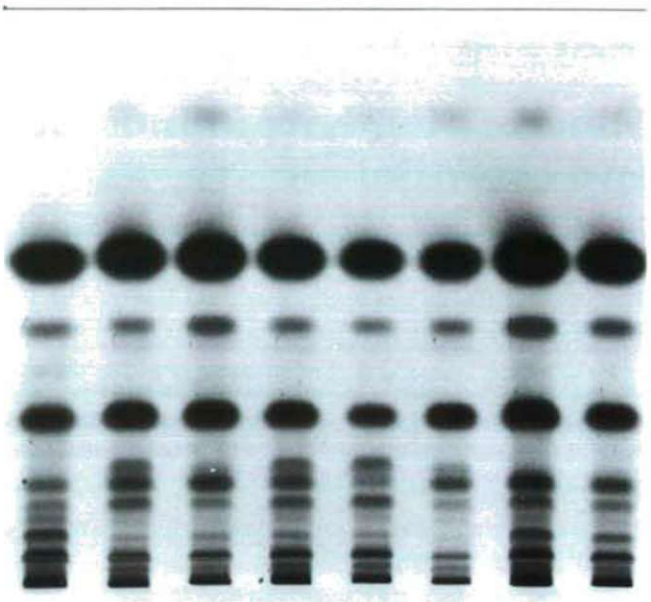

Figure 2. Thin layer chromatogram of sage oil during distillation (chromatography solvent system: toulene-ethyl acetate =95:5, spray reagent: vanillin-sulfuric acid).

Results and Discussion

Elemental content of the samples was measured by ICP-OES and significant differences in the element contcentrations of the samples was found (Table 1). The lithium concentration of leaf and petal of sage samples are relatively very high compared to other medicinal plants (Kabata-Pendias and Pendias 1984; Kabata-Pendias and Mukherjee 2007; Szentmihályi and Then 2007). Relatively high Cr and Li content

Table 1. Element content (mg/kg) in parts of *Salvia sclarea* L.

Elements	Leaf	Bracteol	Fruit	Petal	Calyx-leaf
Al	174.2 ± 5.6	236.1 ± 2.2	9.4 ± 0.21	104.3 ± 1 6	345.5 ± 4.8
B	17.23 ± 1.13	44.01 ± 0.89	17.11 ± 1.02	24.02 ±	36
Ca	11925 ± 125	29165 ± 69	48574 ± 248	6628 ± 164	19169 ± 87
Cr	0.31 ± 0.11	0.51 ± 0.05	0.10 ± 0.01	0.13 ± 0.02	0.19 ± 0.01
Cu	7.1 ± 0.5	8.8 ± 0.2	15.2 ± 0.9	12. 6 ± 1.0	9.3 ± 0.6
Fe	289.3 ± 23	413.9 ± 31.1	43.5 ± 2.4	175.6 ± 9.5	486.6 ± 15.1
K	14758 ± 125	29268 ± 96	10014 ± 85	26058 ± 147	13237 ± 113
Li	9.91 ± 1.02	1.06 ±0.02	3.91 ± 0.21	11.05 ± 0.96	4.24 ± 0.11
Mg	2021 ± 16	4126 ± 54	3110 ± 97	2623 ± 114	3704 ± 99
Mn	14.2 ± 0.56	40. 9 ± 1.41	30.4 ± 1.36	20.1 ± 2.11	32.7 ± 0.98
Mo	0.42 ± 0.01	2.53 ± 0.14	0.73 ± 0.08	0.78 ± 0.03	0.51 ± 0.04
Na	390.2 ± 2.8	340.3 ± 6.8	62.6 ± 1.9	71.3 ± 4.7	390.9 ± 6.4
Ni	1.30 ± 0.05	0.69 ± 0.03	029 ± 0.04	3.11 ±0.021	2.06 ±0.07
P	2624 ± 114	3611 ± 96	6206 ± 75	3101 ± 165	2309 ± 54
Pb	1.91 ± 0.13	3.44 ± 0.31	0.15 ± 0.01	0.21 ± 0.02	4.06 ± 0.02
V	0.60 ±0.02	2.61 ± 0.09	0.14 ± 0.01	0.28 ± 0.01	0.65 ± 0.04
Zn	19.2 ± 1.0	121.5 ± 1.6	40.6 ± 2.7	24.9 ± 1.4	20.4 ± 0.9

Figure 3. Muscat sage oil during distillation.

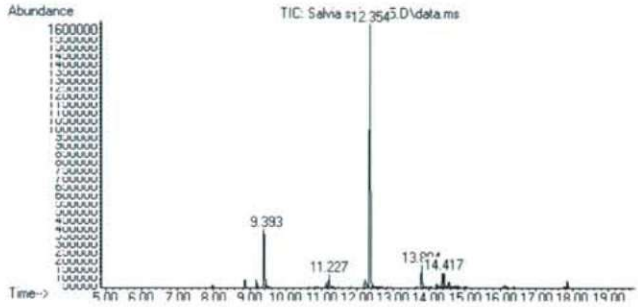

Figure 4. GC spectrrum of muscat sage oil.

was observed in the drug samples wich was published in some medicinal plants earlier (Müller et al. 1988; Then et al. 2004). Sage teas are used for exhaustion and nervosity. The high concentration of lithium of samples compared to the average plant concentration may serve as an explanation for this. Copper and boron content of *Salvia sclarea L.* is also significant, while zinc is accumulated in higher amount in bracteol. In each samples chromium was found in significant amount. The relatively high amount of Pb may be caused by the morphological characteristics of the plant. Trichomas (grandular and covering hair) of the plant surface characterize the muscat sage as it is a common morphological sign of the Lamiaceae family. Trichomes (Figures 1) of the sage may stick the pollutant which could not be removed by washing. Therefore sage samples frequently contain Pb in higher concentration than 2 mg/kg.

The polyphenol content of *S. sclarea* leaf (Table 2.) was in good agreement with the literature data (Szentmihályi et al. 2004; Then et al. 2004).

The volatile oil was obtained from leaf and flowering shoot of sage by steam distillation. The composition of oil of flowering shoot was examined during the distillation. It was obtained that the composition was almost the same during the distillation as we can see according to the thin layer chromatogram in Figure 2. The color of oil is also unchanged during the distillation (Figure 3). By the end of distillation higher amount of oil was obtained from the flowering shoot (Table 3).

The composition of essential oils was analysed by gas chromatographic mass spectrometric method. The qualitative and quntitative composition of leaf and flowering shoot oils

Table 2. Polyphenols content of *Salvia sclarea.*

Plant material		Polyphenol content (%)
S. sclarea	leaf	5.42
	flowering shoot	2.16

Table 3. Essential oil content of *Salvia sclarea.*

Plant material		Essential oil content (ml/100g)
S. sclarea	leaf	0.18
	flowering shoot	0.8

Table 4. Quantitative composition of muscat sage oil according to GC analysis.

Time (min)	Components	Area percentage (%)
9.39	Linalool	13.1
12.22	Carvon	2.5
12.36	Linalyl acetate	61.9
13.07	Geranyl acetate	4.2

Table 5. Element content of flowering shoot and different extracts ± standard deviation (mg/kg) made from flowering shoot of *Salvia sclarea* L.

Elements	Flowering shoot of plant	Essential oil	Plant rest of distillation
Al	124.1 ± 1.2	3.48 ± 2.2	33.1 ± 0.8
B	16.3 ± 0.6	10.71 ± 1.2	6.83 ±0.9
Ca	14582 ± 112	36.99 ± 3.21	7624 ± 100
Cr	4.50 ± 0.14	0.405 ± 0.054	<dl
Cu	128.2 ± 1.5	0.249 ± 0.131	17.98 ± 0.21
Fe	189.5 ± 1.9	2.95 ± 1.28	47.90 ± 4.00
K	23479 ± 159	2.66 ± 1.1	12702 ± 124
Li	26.56 ± 1.02	<dl	0.50 ± 0.09
Mg	2108 ± 5	8.05 ± 2.13	3557 ± 45
Mn	9.46 ± 0.09	0.061 ± 0.026	16.74 ± 0.32
Mo	0.66± 0.23	0.199 ± 0.017	<dl
Na	158.9 ± 7.1	22.26 ± 2.68	2680± 79
P	1750 ± 35	55.04 ± 12.11	3229 ± 15
Zn	18.32 ± 0.34	0.935 ± 0.439	<dl

<dl means under detection limit

are the same, only the percentage occurrences of the components varies. The characteristic gaschromatogram of muscat sage oil is presented on Figure 4.

The main components of muscat sage oil are linalool, carvon, linalyl acetate and geranyl acetate (Table 4).

The element content in flowering shoot (Table 5) is similar as that of the other part of muscat sage (Table 1). The essential

oil also contains elements although in very low concentration. The plant rest of the distillation seems to enrich in Mg, Mn, Na and P.

In summarizing authors investigated the different plant parts of muscat sage herb. It has been stated that the muscat sage samples contains element in similar concentration then other medicinal plants. Relatively high Cr and Li content was observed in the drug samples wich was published in some medicinal plants earlier, nevertheless the Cr content of essential oil is also remarkable.

References

Bernáth J, Dános B, Héthelyi É (1991) Variation in essential oil spectrum of *Salvia* species affected by environment. Herba Hung 30:35-46.

Hungarian PharmacopoeiaVIII. (Ph,Hg. VIII) (2004) Medicina Press, Budapest, pp. 1492-2024.

Illés V, Grosz M, Szebenyi IN (1994) Supercritical extraction. part 2. Recovery of sclareol from muscat sage pellets free from essential oils. Olaj Szappan Kozmetika 43:127-133.

Kabata-Pendias A, Pendias H (1984) Trace Elements in Soils and Plants, CRC Press Inc., Boca Raton, Florida, pp. 1-315.

Kabata-Pendias A, Mukherjee AB 2007) Trace Elements from Soil to Human. Springer, Berlin, Heidelberg, Nem York, pp. 1-550.

Müller A, Diemann E, Sassenberg P (1988) Chromium content of medicinal plants used against diabetes mellitus type II. Naturwissenschaften 75:155-156.

Simándi B, Sawinsky J, Rónyai E, Kemény S, Deák A(1996) Extraction and proccessing of vegetable oil by means of supercritical solvents. Olaj Szappan Kozmetika 45:15-24.

Szentmihályi K, Forgács E, Hajdú M, Then M (2001) In vitro study on the transfer of volatile oil components. J Pramaceut Biomed 21:1073-1080.

Szentmihályi K, Then M, Csedő C (2004) Comparative Study on tannins, flavonoids, terpenes and mineral elements of some Salvia species. Acta Hort (Future for Medicinal and Aromatic Plants) 629:463-470.

Szentmihályi K, Then M (2007) Examination of microelements in medicinal plants of the Carpathian Basin. Acta Aliment 36:231-236.

Szőke É, Kéry Á (2003) Pharmacognosy, Folpress Nyomda Budapest, pp. 1-488.

Then M, Szentmihályi K, Sárközi Á, Szöllősi Varga I (2003) Examination on antioxidant activity int he greater celandine (*Chelidonium majus* L.) extracts by FRAOP method. Acta Biol. Szegediensis 47:115-117.

Then M, Lado C, Szentmihályi K (2004) Comparative study of the mineral element content and phytochemical parameters of of Transylvanian and Hungarian *Salvia officinalis* L. and *Salvia sclarea* L. Olaj Szappan Kozmetika 53:68-69.

Wagner H, Blandt S (1996) Plant drug analysis. Springer, Verlag, Berlin, Heidelberg, New York.

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Connection between measurement of vitamin B_{12} by (RP)HPLC-ICP-MS hyphenated analytical system and antioxidant capacity

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Vitamins constitute a diverse group of organic compounds essential in trace amounts for the proper growth and maintenance of life. They have different specific roles in metabolism, and their lack or excess can generate serious diseases. When it is necessaryTo ensure the adequate intake of vitamins the human diet can be completed with high range of multivitamin tablets and food products supplemented with vitamins, such as B_{12} fortified energy drinks. Each vitamin protects the human body from oxidizing agents, that's why it is of importance to determine the antioxidant capacity and amount of vitamin in food.

The aim of this study was to develop an (RP)HPLC-ICP-MS method for the determination of cyanocobalamin, the compound used for vitamin B_{12} fortification in food. Moreover we also measured the antioxidant capacity and total phenols in the energy drink sample.

In the first part of the work the reversed-phase HPLC separation was optimized with UV detection. After applying various chromatographic set-ups finally an Agilent Eclipse XDB 4.6 x 250mm (5um particle size) column having a C18 stationary phase was chosen. As mobile phases sodium-acetate (pH = 4.0) - acetonitrile and methanol - 0,05 V/V% trifluoracetic acid/ H_2O were used. We worked with two kinds of ICP-MS configuration, - with oxygen gas and without oxygen gas- and these methods were compared.

After the aqueous extraction and alcohol extraction of the energy drink the antioxidant capacity with FRAP assay and total phenol using reagent of Folin-Ciocalteu with spectrophotometer were determined.

The RP-HPLC-ICP-MS system was compared to HPLC-UV system. The selectivity of HPLC-ICP-MS is better, since the cyanocobalamin measurement based on its Co atom content, similarly the sensitivity of HPLC-ICP-MS is hundredfold better, according to the sensitivity of –UV detection. Moreover HPLC-ICP-MS without oxygen gas is more sensitive, then the other, but HPLC-ICP-MS with oxygen gas is occurred to be a more robust system.

The two extractions had a difference in efficiency, because the value of total phenol of alcohol extraction is twofold, as compared to the value of water extraction. The value of antioxidant capacity was too low according to total phenol, which means, that this assay can't measure the antioxidant effect of each phenol compound.

Both of cianocobalamin detection is good for measurement of B_{12} fortified food with simple matrix and vitamin tablet. But the selectivity of the UV detection wasn't enough in the case of energy drink, because the cianokobalamin coeluated with another compound, accordingly we couldn't determine amount of cianokobalamin properly. The ICP-MS detection is more sensitive, according to the UV detection and it measures the cyanocobalamin based on its Co atom content, so determination is more selective. These attributes allow to measure cianocobalamin in very small range, because amount of B_{12} in food is too low (ng/ml).

Phenolic composition and *in vitro* antioxidant activity correlation in *Sempervivum tectorum* extracts

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Sempervivum tectorum (Crassulaceae) is a widely known herb. In folk medicine its juice and leaves were used against inflammation of the ears. The juice was also applied to herpetic eruptions of the skin, minor burns and wounds. It showed remarkably potent antioxidant activity determined by chemiluminometric and EPR spin trapping methods, and inhibited lipid peroxidation induced both enzymatically and non-enzymatically.

Antioxidative, anti-inflammatory and antinociceptive effects of *Sempervivum* has been previously described, though the mode of action is still unexplained and any compounds has not been attributed to these effects. Phytochemical screening of *Sempervivum* extracts with different polarity proved the presence of notable quantity of polysaccharides, polyphenolic

compounds, flavonoids and organic acids. The purpose of this work is the comprehensive chemical analysis of these extracts, implying their fractionation, and the evaluation of their structure-activity relationship.

Extracts of lyophylized and powdered *Sempervivum* tectorum leaves with different polarity (water, 80% (v/v) methanol, methanol, ethanol, aceton, ethyl-acetate and chloroform) has been studied by LC-ESI-MS/MS. Towards a more detailed phytochemical analysis extracts prepared with methanol and chloroform have been fractionated by column chromatographic methods, and the fractions have been studied by LC-MS. *In vitro* antioxidant activity of extracts and fractions has been determined by spectrophotometry using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethyl-benzothiazolin-6-sulfonic acid) radical scavenging activity assays. Antioxidant activity has been characterized by $IC50^{-1}$ values.

The *Sempervivum* leaf extract, prepared by acetonic extraction and acidic hydrolysis had the highest antioxidant capacity. It contained purely flavonoid aglycones and its antioxidant activity was comparable to the one of kaempferol standard. The extract with the second highest antioxidant activity was prepared by 80% (v/v) methanol. Its LC-MS evaluation proved the presence of rutin, kaempferol-di(rhamno)-hexoside and five other kaempferol-glycoside derivatives. Houseleek extracts affected more potent radical scavenging activity against ABTS, than DPPH. Total polyphenolic content and antioxidant activity of *Sempervivum* extracts showed a significant correlation both ABTS and DPPH radicals (r^2=0,907 and r^2=0,967, respectively).

LC-MS evaluation of *Sempervivum* proved to have a high content of flavonoids and other phenolics, which are assumed to play an important role in the eminent scavenging activity of the extracts. On the basis of correlation between total polyphenol content and antioxidant activity of extracts it was concluded, that the remarkable scavenger activity of *Sempervivum* is due to the synergism of its polyphenolic compounds.

Reducing oxidative stress and leukocyte activation in reperfusion injury with controled reperfusion

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Reperfusion of the limbs after acute and persistent ischaemia is associated with high rates of morbidity and mortality despite complete revascularisation. Reconstruction of blood flow will induce reperfusion injury with oxidative stress and inflammatory responses. There are experimental evidences that modification of the initial reperfusion modalities can minimize this reperfusion injury.

In our study we aimed to confirm in an animal model that controled reperfusion (CR) can reduce oxidative srtess and leukocyte activation in reperfusion injury.

In our work we used 10 yorkshire pigs that were divided in two groups. All of the animals underwent a 4 hours infrarenal aortic occlusion: after anethesia we made median laparatomy and clamped the infrarenal abdominal aortae. In the first group after occlusion we removed the clamp, restored the blood flow and closed te wound. In the second group after ischaemia we made CR. CR consisted of 30-minute infusion of a crystalloid reperfusion solution that was mixed with oxygenated blood (the blood:reperfusion solution ratio was 1:1) distal to the occlusion. After this procedure we restored the normal blood reperfusion.

Blood samples were collected before occlusion, on the end of ischaemic period, and after reperfusion in the15th minute (from inferior caval vein), in the 1st and 24th hour, and on 7th day (from peripherial vein). To monitore the evoked oxidative stress superoxide-dismutase activity and reduced glutathion concentration were measured. The degree of lipidperoxidation was marked with the quantity of malondialdehyde. The inflammatory respons was marked with the measurement of leukocyte activation. PMA induced free radical production of the leukocytes was measured.

The lipidperoxidation was significantly lower in the early reperfusion in the CR group. CR also lead to a smaller depletion of the antioxidant enzymes. The speed and rate of free radical production of leukocytes were significantly lower in CR group ($p<0,05$).

The results from this study strongly suggest the hypothesis that the results of conventional embolectomy for acute, severe lower-limb ischemia can be improved by CR. The study was supported by OTKA- K67731.

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Effect of T-2 toxin and different selenium compounds on the glutathione redox and lipid peroxide status of broiler chickens

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Fusarium sporotrichoides is a widespread mould worldwide, producing 'type A' trichothecene mycotoxins, e.g. T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, scirpentriol and diacetoxyscirpenol. Trichothecene mycotoxins affect the antioxidant status of animals, primarily due to their pro-oxidant effect. Selenium, as active site of the Se-dependent glutathione peroxidases plays important role in the biological antioxidant system to protect the adverse effect of harmful free radicals.

The objective of this study was to evaluate the effect of T-2 toxin without and with different forms of selenium on the glutathione redox and lipid peroxide status of 21 days old broiler chickens. The birds were divided into four groups, namely control, fed with T-2 toxin contaminated feed (2.05 mg kg^{-1}) without selenium supplementation ('T-2'), fed with T-2 toxin contaminated feed and supplemented with seleno-methionine (Sel-Plex®, Alltech, 0.3 mg Se kg^{-1} feed) ('T-2+ORGSe') and fed with T-2 toxin contaminated feed supplemented with sodium selenite, Sigma, 0.3 mg Se kg^{-1} feed) ('T-2+INORGSe'). T-2 toxin was produced experimentally on maize by Fusarium sporotrichioides strain NRRL 3299. Five animals were exterminated at the start of experiment as absolute control, followed by extermination of 5 birds from each group at days 3, 7 and 14. Blood, liver, kidney and spleen samples were taken, in which reduced glutathione (GSH), malondialdehyde (MDA) concentration and glutathione-peroxidase (GSHPx) activity were measured.

In blood plasma higher ($P<0.05$) GSH concentration were measured in 'T-2' group compared to control (day 14). In red blood cell haemolysate of the 'T-2' and 'T-2+ORGSe' groups lower ($P<0.01$) MDA concentration was measured compared to control (day 3). In line with this at the same time increased GSH concentration were measured in these groups, which was higher ($P<0.01$) in 'T-2+Se' group than the control. T-2 toxin treatment resulted lower ($P<0.01$) GSHPx activity compared to control and both Se-supplemented groups (day 14). In liver homogenate of the treated groups GSH concentrations exceeded during the whole experiment the values of control one, which was significant in 'T-2' (day 7) and 'T-2 + ORGSe' (days 3 and 7) groups. In the 'T-2+INORGSe' group higher ($P<0.05$) GSHPx activity was measured compared to control (day 7). In kidney homogenate – analogously the findings in liver – elevated GSH concentrations were measured in 'T-2' and 'T2-+ORGSe' groups compared to control at each sampling, which was significant in 'T-2' (day 3) and in 'T-2+ORGSe' group (days 7 and 14). GSH concentration of spleen homogenate in 'T-2' (day 14) group and 'T-2+ORGSe' (day 7) groups were lower ($P<0.01$) compared to control.

According to the results consumption of T-2 toxin contaminated feed (2.05 mg T-2 toxin kg^{-1} feed) for two weeks affects the biological antioxidant system of broiler chickens, increasing the amount and activity of glutathione redox system during the first week of mycotoxin exposure, which efficiently protects the lipids from harmful peroxidation processes. However, in the organs which are play important role of metabolism and elimination of T-2 toxin (e.g. liver and kidney), lowered the amount/activity of glutathione redox system was found in the latter half of the T-2 toxin exposure, causing oxidative stress (as measured by the significantly higher MDA concentrations), while Se-supplementation has beneficial effect in the glutathione redox status.

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Antioxidant steroids and the expression of the gene of superoxide dismutase enzyme

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According to our earlier data most steroid end hormones and some intermediate metabolites of the steroidogenesis are able to inhibit the production of free radical superoxide anion.

In our study we intended to investigate, whether gene-expression of the important antioxidant enzyme superoxide dismutase (SOD) changes after incubation with steroidal compounds.

Peripheral blood samples were collected from healthy volunteers (men and women, aged 20-30 years). After the neutrophil cell separation four different steroid treatments (oestradiol, progesterone, testosterone and cortisol; all in 10^{-8} M concentration, for 2 hours and at 37°C) were performed on 5 million cells. Total RNA was isolated from the treated and control cells, then reverse transcription and real time polymerase chain reaction (RT PCR) were performed on each sample. SYBR Green assays were used for the relative quantification. The SOD_2 gene expression was compared to GAPDH housekeeping gene expression level (incubation with steroidal compounds mentioned above did not alter the expression of this gene in our pilot study).

Upregulated SOD_2 gene expression levels were found after treatment with each steroidal cmpounds. In case of estradiol 14.1fold, progesterone and cortisol 11.3fold increase in average was detected. The largest change (almost twenty –19.7fold rise) was caused by testosterone. The standard deviations of the ddCT values were within one in each treatment.

Based on these data the antioxidant effect of steroid endhormones might be caused at least in part by the enhancement of the SOD gene expression. These results may have innovative pharmacological importance in connection wih free radical mediated disorders.

Metal elements, transmethylating ability and redox homeostasis in tumourous processes

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The receptors, kinases, and nuclear transcription factors affected by metals and metal-induced oxidative stress are associated with cancer growth and spreading. The formaldehyde is in connection with redox homeostasis. HCHO can be formed in transmethylating reactions. Data show the important role of HCHO in proliferative, as well as in apoptotic processes.

Therefore we were interested in studying erythrocyte metal element status, redox homeostasis and transmethylating ability of randomly chosen, operated, middle aged 68 colon and/or prostate tumourous patients and 46 healthy volunteers in both genders.

Tumour markers (CEA, CA 19-9, AFP, PSA) and routine laboratory parameters in sera, redox parameters (scaverger- and reducing ability, SOD, GSHPx) in plasma and erythrocytes, bounded HCHO, HbA1c, protoporphyrin and metal element concentrations in erythrocytes were measured.

We found significant differences in the metal and redox homeostasis between control and operated patients. Significant changes in erythrocyte function can be observed in transmethylating ability and protoporphyrin concentrations as well. The bounded HCHO concentrations were significantly lower in tumourous patients than in healthy controls.

These changes of erythrocytes were similar in operated colon and prostate tumourous patients. We hypothesize that there are similar changes in all other tumour types.

Supporting: ETT 12/2006; ETT354/2006.

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Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Diabetes-associated structural and molecular alterations in capillaries supplying the myenteric plexus in rats

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We have recently demonstrated different susceptibilities of nitrergic neurones located in different intestinal segments to diabetic damage. Their different levels of responsiveness to insulin treatment have also been revealed indicating the importance of the neuronal microenvironment in the pathogenesis of diabetic nitrergic neuropathy. Although the myenteric ganglia are not vascularizated, blood vessels closely related to the ganglia play a key role in creating the proper microenvironment for the ganglia.

Recent data confirm that the loss of the modulatory role of the endothelium may be a critical initiating factor in the development of diabetic vascular diseases. The reduction of the endothelium-dependent vasodilatation is mainly induced by a decreased bioavailability of the endothelium-dependent vasodilator nitric oxide and an increase in the activity of toxic oxygen free radical.

To understand the cellular and molecular background of the diabetes related myenteric neuropathy we investigated the capillaries close to the myenteric plexus and raised two main questions; 1. Is there any difference between controls and streptozotocin-induced diabetic rats in the thickness of the basal lamina surrounding these blood vessels? 2. Is there a direct linkage between the quantitative features of Caveolin-1, which is the major negative regulatory protein for endothelial nitric oxide synthase (eNOS), caveolae and eNOS in the endothelium of these vessels.

In this study a streptozotocin-induced chronical diabetic rat model was used. The rats were divided into three groups: controls, streptozotocin-induced diabetics and insulin-treated diabetic rats. Ten weeks after the onset of diabetes the rats were killed by cervical dislocation, and samples of different gut segments were processed for electronmicroscopical investigations. We measured the thickness of basal lamina by the help of electronmicroscopic morphometry. Postembedding immunohistochemistry was used to study the eNOS and Caveolin-1 expression and interaction in capillary endothels in the vicinity of the myenteric plexus. To evaluate the effects of streptozotocin-treatment and insulin replacement statistical analysis was performed, the probability of $P<0.05$ was set as the level of significance.

In diabetic rats, the endothelial basal lamina what plays a key role in permeability and transendothelial transport was significantly thicker than in the controls. The amount of eNOS and its negative regulator protein, Caveolin-1 was increased in diabetic rats. Immediate insulin replacement significantly prevented the thickening of the basal lamina, and overexpression of eNOS and Caveolin-1.

These results indicate a close relationship between vascular dysfunction and diabetic nitrergic neuropathy, suggesting that endothelial dysfunction in the intestine can be a good prognostic factor for developing enteric neuropathy.

Antioxidant properties of home-made fruit concentrate

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Enhanced oxidative stress develops when production and elimination of reactive oxygen derived compounds (ROS) does not balanced. Enhanced ROS production plays role in development of several diseases. In physiological circumstances elevated ROS production can be decreased by enzymes such as superoxide dismutase, catalase, glutathione reductase, and non-enzymatic ways. One part of the non-enzymatic antioxidants are formed in our body – like serum albumin, coruloplasmine, bilirubin, biliverdin etc. – and other part came from the diet.

During the last decades, several clinical and experimental studies were performed to determine the effects of antioxidant supplementation on health and diseases. However, contradiction in the results considering health prevention were found. Moreover, more and more evidences suggest the pro-oxidant or other disadvantageous properties of mega dose antioxidant supplementation. On these basis, one can assume that the most effective sources of antioxidant are natural origin e.g. the diet itself, and consumption of fresh fruit and vegetables to ensure optimal antioxidant, trace element state mostly advised.

In our country consumption of fresh fruit and vegetables are periodic, therefore, effects of conservation on antioxidant content and properties of products must have been studied. In this study we compared antioxidant properties of several conventional home-made fruit concentrates e.g. jams, and some fruit concentrate made by use of gelatin, which shortened preparation time – and was supposed to keep intact antioxidants and vitamins. We determined in water and methanol extracts of jams the total polyphenol, flavonoid and tocopherol content, and measured capability of fruit concentrate to stabilize DPPH radical (H-donor activity), reduce ferric ion, and inhibit xanthin oxidase activity, which produces superoxide anion.

Dry matter content varied between 30-70%, and gelatinized fruit concentrates had 30-50%. Total-phenol content varied between 0.02-0.15 mg/mg in both water and methanol extract, and flavonoid content was between 0.1-0.8 ug/mg, and was significantly higher in methanol than in water extracts. DPPH stabilization was between 0.02-0.8 ug/mg, ferric ion reduction between 0.17-0.34 ug/mg (both expressed in Trolox equivalent). Inhibition of xanthin oxidase activity was negligible in spite of high flavonoid content. Tocopherol concentration varied within a wide range (alpha-toc: 0-5.9 umol/mg; gamma-toc: 0-0.04 umol/mg; delta-toc: 0-0.2 umol/mg). Ascorbic acid content was not determined. Significant relationship was found between total-phenol content and H-donor activity in both water and methanol extracts. The gelatinized products had the lowest values considering all determined parameter, and traditional plum jam proved to be the best.

In conclusion, traditional method for fruit concentrate (jams) preparation requires long time, however, this method preserved antioxidant content and antioxidant properties of jams, since it ensure relative low temperature during the whole process. Artificial gelatinization had disadvantageuos effect in all studied parameter.

Connection between different raising system and antioxidant parameters in geese

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The profitability of animal production largely depends on how efficiently the animals utilize the feedstuffs for maintenance and production. This is markedly influenced by nutritional and keeping systems during the rearing period of animals. The energy and crude protein contents of the diets, and stocking density have great influence on metabolic processes, hormonal status and the redox system of the animals.

The physiological effect of different management techniques used in geese production is scarcely investigated. Several reports have been presented recently describing the biochemical effect of stocking density, however no literature data have been published concerning the influence of stocking density on antioxidant system of geese. The objective of this study was to investigate the connection between different nutritional and management technologies and antioxidant parameters of geese determining two plasma parameters.

540 Gourmaud liver type hybrids (representing both sexes) were included in the experiment, from 1 day to 64 day of age. At the start of the experiment two different stocking densities were used: 2.5 geese/m^2 (12 geese/cage) and 1.5 times higher, 3.8 geese/m^2 (18 geese/cage). Geese were fed with experimental diets with 11, 12 and 13 MJ/kg ME (low, medium and high) each contained 18, 20 and 22% CP in the starter, 16, 17.5 and 19% CP in the grower and 14, 15 and 16% CP in the finisher. Blood samples were taken from wing vein at the end of trial (9 wk of age). The tested plasma parameters for antioxidant status of geese were chemiluminescent intensity (CI) and radical scavenging capacity (RSC). Chemiluminescence assay in plasma was carried out by the method of Blázovics et al. (1999). Radical scavenging capacity of feed samples was determined in the presence of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as described Blois (1958).

The dietary metabolizable energy content had significant effect on antioxidant system of geese. Geese fed diet with highest energy content had increased radical scavenging capacity and the value of chemiluminescent intensity was the best. There was no connection between dietary crude protein content and the measured antioxidant parameters. The redox parameters were similar in male and female geese, there was no sexual differences. The stocking density had significant effect on radical scavenging capacity, however chemiluminescent intensity did not differ. At a stocking density rate of 12 birds per pen plasma radical scavenging capacity was significantly higher, 0.352 mmol/l, compared to 0.299 mmol/l value found in pen with 18 birds.

By measuring these two parameters characterizing the redox status of the organism, significant relation could be revealed between the nutrition and management and the antioxidant system of the geese. For a more detailed interpretation of this effect, further parameters related with main elements of the antioxidant system would need to be analysed. Such data could help to find adequate compounds as feed additives supporting the antioxidant system

This work was supported by the National Office for Research and Technology (OM-00160/2007).

The effect of the fluid resuscitation method and the n-acetylcysteine supplementation on the oxidative stress response after severe burn injury

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The hypovolaemia, followed by burn injury, induces severe oxidative stress in the body. Our previous study has proven that intrathoracic blood volume index (ITBVI) is a better target parameter of fluid resuscitation, than hourly urine output (HUO). There are only few data in the literature regarding to the effectiveness of antioxidant therapy after burn injury. The aim of our study was to analyze the effect of the fluid resuscitation method and the n-acetylcysteine (NAC) supplementation on the oxidative stress response after severe burn injury.

Twenty-seven patients were involved to our study. In Group I (n=8) the fluid resuscitation was guided by the HUO, in Group II (n=8) by the ITBVI. In Group III (n=11) the ITBVI guided fluid replacement was supplemented with NAC administration during the study period. Venous blood samples were taken from patient on admission and on the next 5 consecutive days. We measured the blood-, and oxidative stress parameters (malondialdehyde (MDA), reduced gluthation (GSH), protein sulfhydril (PSH) groups in plasma, the activities of superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO) enzymes, and PMA induced free radical generating capacity (ROS). Blood samples from healthy volunteers (n=9) served as the control.

There was no significant difference between the groups regarding to age and burned body surface. There was a higher survival in the NAC treated group. White blood cell count normalized by the 3rd day in all groups, but the relative number of granulocytes was significantly ($p<0.05$) higher, the relative number of lymphocytes was significantly ($p<0.05$) lower in the HUO Group. The marked granulocytosis and lymphocytopenia were on the mend in the NAC Group. The MDA level was elevated ($p<0.05$) all along, the ROS from the third day ($p<0.05$) during the observation period compared to the Control Group. MDA in the plasma was lower, the ROS was higher in the NAC Group. The GSH and PSH level, as well as the SOD activity was significantly lower ($p<0.05$), the CAT activity was significantly higher ($p<0.05$) in the HUO and ITBVI Groups compared to the Control Group. There was no significant difference between the patient groups. NAC supplementation significantly increased the PSH levels and the GSH level normalized earlier. The NAC treatment had no effect on the SOD and CAT activity compared to the ITBVI Group.

The ITBVI guided fluid resuscitation has a beneficial effect on the prooxidant state of the body, but it has no effect on the prooxidant parameters. The adjuvant NAC treatment improved the survival of the patients, increased the endogenous, non-enzymatic antioxidant capacity, but didn't reduce the prooxidant parameters and the activation of the white blood cells.

The work was supported by the OTKA K060227 grant.

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http://www.sci.u-szeged.hu/ABS

Biglycan protects cardiomyocytes against simulated ischemia/ reoxygenation injury via an NO-dependent mechanism

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Although biglycan, a proteoglycan component of extracellular matrix, has been suspected to contribute to the development of atherosclerosis, overexpression of biglycan has been shown to induce cardioprotective genes including nitric oxide (NO) synthases in the heart of a transgenic mouse model.

The aim of the present study was to test whether biglycan is cardioprotective against hypoxia/reoxygenation injury in cardiomyocytes and if an NO-dependent mechanism is involved in the cytoprotection.

Therefore, primary cardiomyocytes were prepared from newborn Wistar rats and kept in growing medium (90% DMEM, 10% fetal calf serum) under normoxic conditions (37°C, 5% CO_2). Two days old cultures were treated with 1, 3, 10, 30 and 100 nM biglycan. In separate experiments, biglycan (30 nM) was combined with the NO synthase inhibitor L-nitro-arginin-methyl-ester (L-NAME, 100 μM). After a 20-hour pretreatment, media of the cultures were replaced with a "hypoxic" solution and plates were kept in a hypoxic chamber (gased with 95% N_2 and 5% CO_2 at 37°C) for 150 minutes, which was followed by 120 minutes of reoxygenation. All treatments were continued throughout hypoxia and reoxygenation. Finally, viability tests were done in all groups with Trypane blue staining. In order to check the effect of biglycan on NO synthase (NOS) expression, in separate experiments, normoxic cells were treated with 30 nM biglycan for 20 hours and then mRNA and total protein were isolated.

After simulated ischemia and reoxygenation, 41.8±1.0% of the cells died in control cultures. Biglycan significantly decreased cell death at 3, 10, 30 and 100 nM concentrations. Protection was the strongest at 30 nM (17.3±2.4%). Biglycan enhanced expression of mRNA of endothelial NOS, but not inducible NOS. Endothelial NOS expression at protein level was also significantly elevated after biglycan treatment. The L-NAME abolished the cytoprotective effect of biglycan (36.3±1.6%).

The proteoglycan biglycan exerts a cytoprotective effect against hypoxia/reoxygenation injury via at least in part an NO-dependent mechanism.

Antioxidant characterization of perspective apricot hybrids

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Health-promoting effects of fruits are at least partially attributed to the antioxidant compounds accumulating in fruit flesh. Apricot fruit contains three major types of antioxidant compounds: water-soluble ascorbic acid (vitamin C), lipid-soluble carotenoids and polyphenolics encompassing both hydro- and lipophilic components. To survey the potential health-effects of apricot it is important to know about the variations in quantity of the antioxidant compounds present in fruit. Studies on parents and their progeny may help to shed light on the inheritance of fruit antioxidant properties and to clarify if the increase in fruit antioxidant capacity may be possible in a carefully designed breeding program.

The measurements were carried out on the apricot cultivars maintained in the germplasm collection of the Department of Genetics and Plant Breeding, CUB and 18 hybrids obtained from a breeding program of the Department of Genetics and Plant Breeding, CUB. The following parameters were studied in apricot fresh fruits: colour values (lightness factor, hue angle and chroma colour); ferric reducing ability (FRAP); DPPH-radical scavenging activity; total radical scavenging capacity measured with chemiluminescence methods; as well as total phenol (TPC) and vitamin C contents measured with spectrophotometer and HPLC-DAD, respectively.

The FRAP and TPC assays revealed 22- and 21-fold differences, respectively, between the lowest and the highest values, indicating a great diversity in the antioxidant power of apricot fresh fruits. A perspective hybrid produced outstanding values in all of the antioxidant assays, exceeding 2.5-times the same parameters determined for the best commercial cultivar. The

FRAP values of twelve hybrids resulting from the cross 'Bergeron' ×'Baneasa 4/11' varied between the values determined for the parents ('Bergeron': 3.57 mmolAA/L and 'Baneasa 4/11': 1.12 mmol AA/L). Three hybrids showed FRAP values very similar to that of the 'Baneasa 4/11', while two others almost reached the level measured in 'Bergeron'.

The closest correlation occurred between the FRAP and DPPH-radical scavenging capacity. Close correlations were also obtained between FRAP, TPC, DPPH and vitamin C content data. Colour values did not show significant correlations with any of the measured parameters of water-soluble antioxidants, since colour values were correlated exclusively with the lipid-soluble carotenoids.

Our results indicate that several valuable genotypes can be selected from a progeny obtained from crosses where at least one of the parents is characterized by enhanced fruit antioxidant properties.

This work was co-financed by the Ányos Jedlik programme NKFP06A2-BCETKA06 and the NKTH-OTKA K68921 grant.

Regulation of metal responsive transcription factor MTF-1 expression in common carp

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Metal responsive control of gene expression allows organisms to adjust the concentration of essential metal ions such as Zn^{2+} and Cu^{2+}, within an acceptable range and cope with detoxification of heavy metals (Cd^{2+}, Pb^{2+} and As^{3+}) with no biological function. Metallothioneins (MTs) are widely inducible at transcriptional level by a variety of metals and other stress conditions such as accumulation of reactive oxygen species, hormones and cytokines. Transactivation of metallothionein genes involves the Metal-responsive Transcription Factor (MTF-1) a metal responsive element (MRE) binding, zinc sensitive protein.

In this study we present the first evidence for an *mtf-1* splicing variant (*mtf-1.1a*), originated from the brain of unstressed common carp. We have follow the level of *mtf-1.1a* mRNA in the liver, kidney, heart, muscle and brain of unstressed animals and the effect of heavy metal loading (Cd and As) on the alternative splicing of *mtf-1.1* transcript. For the detection and semiquantitative determination, an *mtf-1.1*-specific primer pair was designed. This primer pair has the potential to amplify a segment from both *mtf-1.1* and *mtf-1.1a* in the same PCR reaction, with a well-distinguishable size difference.

The splice variant of *mtf-1.1* mRNA codes for a truncated MTF-1.1 protein. The lack of a 103 nucleotides internally in the *mtf-1.1a* transcript, between positions 1047-1149, results in a frame shift causing an early termination of translation. The putative MTF-1.1a protein consists of the first 349 amino acids of MTF-1.1 followed by an additional 64 amino acids, which don't resemble at all to the corresponding region of MTF-1.1. The 349 amino acid covers the six Zn-finger DNA binding domains, the nuclear localization (NLS) and the nuclear exporting (NES) signals and the first 12 amino acid of the acidic region. Under unstressed conditions *mtf-1.1a* was detected in all tissues examined, but the liver, with the highest level in the brain. Arsenic alters the level of both *mtf-1.1* and *mtf-1.1a* transcripts in an isoform- and tissue-specific manner. Cadmium had no measurable effect on the alternative splicing of *mtf-1.1* in the liver, while the amount of both *mtf-1.1* transcripts gradually decreased in the brain.

The above observations suggest a tissue- and stressor-specific function of the predicted MTF-1.1a protein. In addition, we have already identified another MTF-1-coding gene, *mtf-1.2*, exclusively expressed in the brain of unstressed animals. The possible presence of 3 MTF-1 protein variants in the brain suggests a crucial role of the MTF-1s in this organ. MTF-1.1a might function as a negative regulator of MRE-controlled expressions. However, it is also possible that the introduction of a new C-terminal domain might result in a new level of regulation by recruiting new proteins to MTF-1.1-controlled promoters.

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Investigation on biological action of Small-flowered Willowherb

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Small-flowered Willowherb (*Epilobium parviflorum* Schreb.) is traditionally used in the prevention and complementary treatment of benign prostatic hyperplasia (BPH). BPH is maintained by complex and still not entirely discovered pathological factors, but age-related hormone imbalance, decreased apoptosis, inflammation and excessive oxidative stress are definitely involved in the patomechamnism. Based on our analysis, willowherb is rich in various structured phenoloids. Its most characteristic compounds are myricetin-, quercetin- and kaempferol-glycosides, as well as the often macrocyclic derivatives of ellagic- and gallic acid (e.g.: oenothein B). Apolar extract of willowherb contains β-sitosterol.

Our workgroup aimed to investigate the mechanism of action of *Epilobium parviflorum* with special regard to its antioxidant activity and anti-inflammatory effect.

H-donor capacity of willowherb was measured by two different spectrophotometric methods (ABTS, DPPH). Inhibitory action on lipidperoxidation was examined with TBA assay, on bovine brain liposomes. Antioxidant cell-protective effect of *Epilobium* was studied on fibroblast cells. Anti-inflammatory effect was investigated on macrophage cells, by examination of COX-enzyme inhibitory action of extract.

Willowherb showed a remarkable H-donor activity (EC_{50}: ABTS 1.71 ± 0.05μg/ml; DPPH 3.01 ± 0.03 μg/ml), comparable to that of ascorbic acid and trolox. In the TBA assay willowherb extract showed concentration-dependent inhibition of lipid peroxidation at doses over 0.20mg/mL (IC_{50}=2.37 ± 0.12mg/mL). *Epilobium* extract exerted steady and concentration-dependent protective effect against oxidative damage generated on fibroblast cells. The protective action was comparable to that of catalase enzyme (250IU/ml). *Epilobium* extract showed concentration-dependent COX-enzyme inhibitory action (IC_{50}=1.4±0.1μg/ml).

Biological action of *Epilobium parviflorum* has been *in vitro* investigated. Based on our results, willowherb possessed high H-donor capacity and antioxidant cell-protective effect. The extract inhibited lipidperoxidation and the activity of COX-enzyme. These results suggest that extract of *Epilobium parviflorum* has antioxidant and anti-inflammatory properties which are likely to contribute to its beneficial effect in BPH. However, for wider, evidence-based application of willowherb further in vitro and in vivo studies are necessary.

Pigment photosensitized reactions make dark-grown pea epicotyls wilt in the light – direct detection of ROS promoting type-I and type-II photochemistry

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Upon illumination, epicotyls of dark grown pea (*Pisum sativum* L.) seedlings loose turgor in their middle section and wilt. Direct detection of singlet oxygen shows the involvement of type-II photoreactions, while co-localization of hydrogen peroxide and protochlorophyllide monomers suggests the contribution of type-I photodynamic pigment reactions as well. Hydroxyl radicals were detectable with spin trapping electron paramagnetic resonance spectroscopy and were also triggered by adding hydrogen peroxide in the dark, demonstrating Fenton chemistry.

In plants, native arrangements of pigment-protein complexes are critical during early plant development. In most angiosperms, various chlorophyllous pigments are safely stored in aggregates (macrodomains), such prolamellar bodies of leaf etioplasts, to prevent photo-oxidation. However, etioplasts in epicotyls or other stem related organs contain only few and small macrodomains and the chlorophyll precursor pigments, such as protochlorophyllide (Pchlide) are predominantly in monomer state in them.

Seven days old pea (*Pisum sativum* L.) epicotyls were germinated and grown in darkness. Singlet oxygen ($^1O_2$) production was visualized with DanePy (3-[*N*-(β-diethylaminoethyl)-*N*-dansyl] aminomethyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrole) fluorescence. H_2O_2 was detected by DAB (3,3'-diaminobenzidine) staining or by transition electron microscopy (TEM) using $CeCl_3$. Hydroxyl radicals (•OH) were detected with spin trapping EPR spectroscopy using POBN or on the basis of HTPA (hydroxyl-terephthalate) fluorescence. Protochlorophyllide (Pchlide) localization was detected by fluorescence microscopy, its monomers/oligomers were identified by low temperature fluorescence emission spectra.

Illumination caused fast turgor loss and wilting in middle segments of the epicotyls accompanied with accumulation of water in the intercellular cavities. During this process, porphyrin-type pigments were gradually bleached, while $^1O_2$ and lipid peroxidation products were detected suggesting a type-II, porphyrin (Pchlide or Chlide) -photosensitized mechanism.

On the other hand, selective assays showed the presence of three different ROS: $O_2^{-\bullet}$, H_2O_2 and •OH in the illuminated pea epicotyls, preferentially in the mid-sections. H_2O_2 was mainly produced along the radial walls of cells in areas also rich in monomer Pchlide. Although •OH production, which was restricted to the mid-section was light-dependent, the $H_2O_2 \rightarrow$ •OH conversion also occurred without illumination, showing the presence of Fenton-catalysts in this region. These data demonstrate that products of a type-I photoreaction also contribute to disordered water status of the epicotyls and their wilting in light.

TIP47 protects of mitochondrial membrane integrity and inhibits oxidative stress-induced cell death

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The intracellular role of tail-interacting protein of 47kDa (TIP47/PP17b) has been controversial, no data are available for its possible role in tumor development, or in regulation of cell death. TIP47 is expressed in almost if not all tissues. During pregnancy, TIP47 serum levels increase; after birth they drop. Although some TIP47 is found on lipid droplets, it is known to be required for the delivery of mannose 6-phosphate receptors from late endosomes to the Golgi, both *in vitro* and in living cells. The protein binds the cytoplasmic domains of the cation-dependent and cation-independent receptors, and is recruited to late endosomes by binding to Rab9 GTPase. The loss of TIP47 destabilizes Rab9 which is also required for proper receptor transport.

The aim of this study was to find an intracellular role of this protein.

The vector containing TIP47, trunceted-TIP47 or the empty pcDNA3.1 vector was transfected into NIH3T3 cells. Cells were treated with H_2O_2 and cell viabilities were measured by MTT-viability assay. TIP47 was silenced by diced-siRNA in Hela cells. Mitochondrial membrane potential was monitored on isolated rat liver mitochondria in vitro by fluorescence of Rh123 or on TIP47 transfected and treated NIH3T3 cells in vivo. Depolarization of mitochondria can be visualized *in vivo* by using the membrane potential sensitive dye, JC-1 by fluorescent microscopy. Ratio of apoptosis and necrosis were evaluated after double staining with fluorescein isothiocyanate (FITC)-labeled annexin V and propidium iodide using flow cytometry and fluorescent microscopy.

TIP47 was over-expressed in a cell line normally not expressing it (NIH3T3), or suppressed by small interfering RNA in a cell line that normally express TIP47 (HeLa) at high extent before exposing cells to oxidative stress. Over-expression of TIP47 prevented hydrogen-peroxide induced cell death and the collapse of mitochondrial membrane potential. Suppression of TIP47 synthesis by small interfering RNA technique sensitized the HeLa cells to hydrogen peroxide induced cell death. We proved with both in vitro and in vivo experiments that TIP47 caused hyperpolarisation of mitochondrial membrane and reduced Ca^{++} induced mitochondrial membrane depolarization. In view of the fact that mitochondria may role in both apoptotic and necrotic cell death, we used flow cytometry to determine the percentage of apoptotic and necrotic cells. The results unambiguously justified protective effects of TIP47 protein.

We provided evidence that TIP47/PP17b can bind to mitochondria and can protect mitochondrial membrane integrity, as well as can prevent oxidative stress induced cell death providing the first evidence the possible oncogenic property of TIP47/PP17b.

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Acta Biologica Szegediensis
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Oxidative stress in Parkinson disease

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Parkinson disease (PD) is a slowly progressive degenerative neuronal disorder comprising combinations of motor problems like bradykinesia, tremor-at-rest and muscle rigidity. Its major characteristic is the selective degeneration of the nigrostriatal pathway. PD is a multifactorial disease; both genetic and environmental factors could play important role in its pathogenesis. From environmental factors the mitochondrial complex I. inhibitor pesticide rotenone has remarkable significance. PD and other chronic neurodegenerative diseases which are characterized by a selective loss of distinct groups of neurons, have a common patomechanism, since oxidative stress and dysregulation of transmitter release play a central, but not initiative role in the development of the disease. One of the most effective therapy target is the monoamin oxidase, besides some compounds with antioxidant properties are seem to be neuroprotective in experimental PD model. The ideal drug decreases the level of pathological free radical production as a monoamin oxidase inhibitor, and via its antioxidant capacity, it also decreases the level of already existing reactive intermediers.

Oour goal was to identify compounds combining these two properties, thus having significantly higher neuroprotective effect than the currently used drugs.

We set an *in vitro* system to screen numerous multitarget drug candidates. PC12 cells were treated with rotenone, the protective effect of the various compounds on cell survival was determined.

From the reference drugs we found significant neuroprotection by deprenyl and rasagiline, and severe neurotoxicity by L-Dopa. Up to this point the majority of the tested compounds did not achieved the level of neuroprotection rendered by deprenyl or rasagiline. However some compounds attained significant neuroprotection in the rotenone model.

The screening of numerous compounds can be realized quickly and dependably with the *in vitro* Parkinson model. It is suitable for identifying drug candidates that have even greater neuroprotective effect, than the currently used drugs.

Eexamination of oxidative stress markers and liver function after open- and transgastric small bowel resection

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In Natural Orifice Transluminal Endoscopic Surgery (NOTES) a flexible endoscope is passed through a natural orifice (transgastric, transvaginal, transanal, transvesical) of the body and intra-abdominal procedures can be performed. Experimental data shows that this new procedure can reduce surgical trauma and inrtaabdominal adhesion formation is minimal. The technique without visible scar launched a new trend in surgery, which is no-scar surgery.

The aim of this study was to compare surgical trauma after open- and transgastric small bowel resection.

Within the framework of EURO-NOTES research program, with co-workers of Markus-Krankenhaus Surgical Clinic (Frankfurt am Main, Germany) transgastric (TG=7) and open small bowel (O=6) resection was performed on pigs. Oxidative stress marker concentrations (malondialdehyde (MDA), glutathione (GSH), SH-groups (SH-), superoxide-dismutase (SOD), liver enzyme (glutarate-oxalacetate-transzaminaze (GOT), glutamate-piruvate-transzaminaze (GPT), laktate-dehidrogenaze (LDH), gamma-GT (GGT), alkalic-phosphatase (ALP)) and total bilirubin (SeBi) concentrations were measured. Blood samples were taken before operation, at the end of operation, on first, third and on seventh postoperative day for biochemical tests.

There were no complications during surgery, all pigs survived. Oxidative stress marker concentrations were increased after operations in both group and decreased postoperatively. GOT, GPT, LDH, SeBi concentrations were increased after operation and decreased postoperatively in both groups. GGT and ALP concentrations were decreased during on monitoring days also in both groups. There was no significant difference between the two groups in concentration.

Transgastric approach means similar surgical stress like open technique. Further examinations are needed with a larger number of pigs and sensitive parameters.

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Acta Biologica Szegediensis
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Comparative analysis of vitamin content of food supplements marketed in Hungary

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According to the Order of Hungarian Minister of Health No. 37/2004. (IV. 26.) food business operators must notify their food supplements at National Institute for Food and Nutrition Science before placing them to the Hungarian market. National authorization before marketing of food supplements ended at first of May 2004. Producers of food supplements have been allowed to use vitamins and minerals in chemical forms exclusively given in supplements I. and II. of the Decree mentioned above. Since 1st of May 2004 more than 4500 food supplements have been notified in Hungary. This number increases daily by four but in the first four months of 2009 nearly nine products per day were included into the list of notified products. More than half of the notified products contain vitamins, solely or in combination with other vitamins, minerals, plant extracts or isolated substances, as well. Most frequently used vitamins are C, E and Bs, including B1, B2, niacin and B6, at smaller frequency other vitamin Bs, vitamin A, D and K are the components of food supplements. Products contain vitamins at different levels but majority of them have vitamins not more than RDA (recommended dietary allowances). Controlling of the real composition of the products before marketing depends only on the decision of the food business operators; the market control authorities screen the products only at limited frequency.

In the frame of PHARE 2005/17/520.01.01 transition facility project National Institute for Food and Nutrition Science was provided with a Thermo Surveyor Plus HPLC-DAD/FLD equipment which could serve as the tool for the screening of the level of vitamins in food supplements sold in Hungary. Validated methods were set out for separating, qualifying and quantifying water and fat soluble vitamins with different chemical structure in food supplements. The most important element of the methods was the sample preparation step (direct extraction, saponification, or acid base solution) of products with different forms as hard and soft gel capsules, tablets, and so on, for obtaining the whole amount of active substances. The preparation step was followed by the separation of different vitamins and chemical forms on a reversed phase chromatography column and finally detection based on UV signal of the molecules. With the use of newly developed methods screening of vitamin content of about fifty food supplements found in Hungarian market was done. It can be stated that most part of the products contain vitamins at level indicated on the label, only in certain cases significantly higher or lower level (20%) of vitamins could be detected. There were some products where declared amount of vitamin indicated in the label did contain only the added vitamin and amount coming from natural source was not summed up. Methods developed are suitable for separation, qualifying, as well as quantifying vitamins with different chemical structure and monitoring the composition of food supplements marketing in Hungary.

Exogenous selenium influences the reactive oxygen radical production and restores intestinal perfusion in a porcine model of cardiac tamponade

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Selenium (Se) is essential for the function of redox regulator enzymes that have major roles in cardiovascular diseases with transient hypoxia, but the clinical value of Se replacement is still controversial.

The aim of our study was to assess the effects of Se treatment on reactive oxygen intermediates (ROI) production and splanchnic circulatory consequences in experimental cardiac tamponade (CT).

Anesthetized, thoracotomized minipigs (n=6) were subjected to acute CT by intrapericardial fluid infusion; the mean arterial pressure was kept at 40 mmHg for 60 min. After removal of the pericardial fluid, macrohemodynamic changes, small intestinal flow and pCO_2 gap (tonometric probe), blood ROI (superoxide and H_2O_2 production, chemiluminometry), plasma

nitrite/nitrate (NOx) level (Griess reaction) were monitored for 180 min. Another group of animals (n=6), received Se infusion (25 μg/kg/h iv) after CT induction.

CT was followed by hemodynamic signs of cardiogenic shock. During resuscitation, the significantly increased intestinal pCO_2 gap, elevated ROI production of the blood referred to prolonged mesenteric ischemia in spite of restored macrohemodynamics. In contrast, superoxide producing capacity of blood, NOx production in plasma, intestinal blood flow and pCO_2 gap were significantly improved by Se treatment.

CT-caused peripheral circulatory derangement could be effectively influenced by Se treatment due to reduced free radical production and improved intestinal microperfusion.

Study on total phenol content and antioxidant capacity (FRAP) of Ginkgo biloba L. leaves from different places

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Blood flow regulating and antioxidant effects of ginkgo (Ginkgo biloba L.) leaves are well known. Products containing standardized ginkgo leaf extracts are among the most popular medicinal goods in Hungary. Also more and more ginkgo teas (crude leaf drugs) are available in the trade flow as monoteas or in mixtures.

Total phenol content and antioxidant capacity (by the FRAP method) were determined from extracts of ginkgo leaves collected in different places.

Places of collection (all in Hungary): Budapest city (Botanical Garden of Eötvös Lóránd University called "Füvészkert", Botanical Garden of Corvinus University of Budapest - BCU, Margit boulevard in the city centre), Gödöllő city (park of Szent István University), Paks city (city centre) and Székesfehérvár city (city centre). In Füvészkert we collected leaves both from male and female trees . Leaves were dried at 30°C and than pulverized. Based on prescribes of the Hungarian Pharmacopoeia aqueous and aqueous ethanolic (water/ethanol 80/20, v/v) extracts were made from the prepared leaves. Total phenol content was measured spectrophotometrically (λ = 760 nm) with the use of Folin-Ciocalteu reagent. Antioxidant capacity was determined also spectrophotometrically by the FRAP method.

In case of all samples total phenol content of aqueous extracts was higher than that of aqueous ethanolic extracts. For aqueous ethanolic extracts more pronounced differences were obtained among the samples than for aqueous extracts. In case of aqueous extracts the highest total phenol content (0,132 mg/ml) was detected in the sample from the tree of BCU, while the trees of Füvészkert hed the lowest value (0,079-0,081 mg/ml). In aqueous ethanolic extracts the total phenol content was the highest (0,089 mg/ml) in ginkgo leaves collected in Gödöllő city, statistically signinicantly higher than in the other samples. In case of ethanolic extraction big differences between the sexes could be detected for the phenol content. The lowest value (0,016 mg/ml) was detected from the sample of the female ginkgo tree of Füvészkert.

Total antioxidant capacity determined by the FRAP method was higher in aqueous extracts than in aqueous ethanolic extracts. In case of ethanolic extraction samples of old trees of Füvészkert showed an unexpectedly low antioxidant capacity (0,21-0,28 mmol ascorbic acid/l), significantly lower than the other samples (0,63-1,22 mmol AA/l). In aqueous ethanolic extracts the highest antioxidant capacity (0,81 and 0,73 mmol AA/l) were found for samples of Gödöllő city and of BCU, while the lowest (0,31 mmol AA/l) for the sample of Székesfehérvár city. Antioxidant capacity values of the other samples were about the same. Antioxidant capacity of leaf extracts of male and female trees did not differ from each other.

Both of total phenol content and total antioxidant capacity were higher in aqueous extracts than in aqueous ethanolic extracts. Among the samples collected from different places significant differences were obtained for both of the investigated parameters. Differences in antioxidant capacity did not show connection with the pollution grade of sampling places, these could be caused by age of the ginkgo trees. However the significant differences may worth consideration, as it could have an important role in the therapeautic use of ginkgo leaves. It is also worth to mention, that the antioxidant capacity of ginkgo leaf extracts was much lower than that of products containing standardized extracts, so antioxidant effect of ginkgo teas is lower than that of the standardized extracts.

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Study the structure-activity relationship of silybin analogues using different ROS production sources

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Reactive oxygen species (ROS) formation is indispensable for life. They play role in optimal working of immune system (killing mechanism), in different signal transduction processes, induction of apoptosis or activation of genes. Elimination of ROS requires presence of enzymatic and non-enzymatic antioxidants, in which natural origin antioxidants from the diet are involved. Overproduction of ROS initiates development of diseases; therefore, antioxidant supplementation was suggested for prevention or treatment of those states. However, results of these studies were contradictory, such as the role of Vitamin E in prevention of cardiovascular diseases which was mainly due to the fact that, in most cases, antioxidant requirement e.g. original Vitamin E level, and source(s) of ROS was not determined or took into account.

To certify the importance of ROS source in antioxidant activity of a given molecule, several silybin (1) analogues were synthesized namely, flavanon- (2, 3), flavone-derivatives (4, 5), flavanolignan skeleton (6), dehydrosilybin (7), and hydnocarpin (8), and their effects were compared in inhibition of superoxide anion production in phorbol-ester stimulated human neutrophils, xanthine oxidase activity, H-donor activity, LDL oxidative resistance, and ferric ion reducing capability.

It was found that only silybin and dehydrosilybin possessed measurable H-donor activity. Ferric ion reduction was observed in case of silybin (0.68 teq), dehydrosilybin (0.45 teq) and hydnocarpin (0.28 teq). Xanthine oxidase activity was inhibited by silybin and its flavanon analogues (2, 3) in similar extent (IC50~32 uM), and flavone analogues of silybin (4, 5), dehydrosilybin and hydnocarpin were more effective (IC50~0.2 uM), and silybin skeleton (6) was absolutely ineffective. Oxidative resistance of LDL increased by 3.2 fold by silybin, 2.7 fold by its flavone analogues, while the other compounds had weaker effects (1.2 fold), and silybin skeleton was ineffective. However, phorbol-ester stimulated superoxide anion production was inhibited most effectively by silybin skeleton (58%), followed by hydnocarpin (52%), dehyrosilybin (50%), and flavone analogues of silybin (40%), respectively. Silybin itself and its flavanon analogues were the less effective (15-22%). The inhibition in superoxide anion production was due to inhibition of PKC-alpha activation in neutrophils. On this basis we can conclude that 1. Inhibition of xanthine oxidase activity requires OH groups, and presence of a double bond in C ring enhances the effect; 2. Ferric ion reduction required the intact flavanolignan structure, and double bond in ring C attenuated the effect; 3. Flavanolignan structure independently the presence of OH groups, and double bond had low H-donor activity; 4. Oxidative resistance of LDL was modified by silybin and double bond in ring C attenuated its effect; 5. However, effective inhibition of PKC in human neutrophils was achieved in case of silybin skeleton, e.g. in the absence of OH groups, 2-methoxy-4 hydroxy-phenyl, and 3-hydroxymethyl groups, in the presence of one or more side chain(s) attenuated the effect of silybin skeleton.

In summary, these results clearly show that relationship between structure and antioxidant capacity of a given antioxidant is depend strongly on type(s) of ROS source. Therefore, we suggest the identification of ROS source before initiation of antioxidant supplementation therapy.

Reaseach was sponsored by Fund of Hungarian Academy of Sciences (T 42550).

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Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Chromatographic analytical opportunities on a thin film of mobilizable methil-groups of different biological objects under the influence of exogenic treatment

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In case of hyperlipidemic disease caused by alcohol, due to the S-Adenosyl-Methionin (SAM) deficiency, several vital metabolic roads, like protein synthesis, the synthesis of chatecholamins, and the nucleic acids, the methilation of phosphatidil-ethanolamin and phosphatidil-choline, and the activity of the synthesis of glutation, by which lipid peroxidation is hampered, decrease.

It has been proven that SAM is the methyl-donor in the trance-methyl reactions, and that the enzymatic methylation / demethylation processes equally generate HCHO.

Beside the free radicals and H_2O_2, the HCHO plays an important role in living organisms. To better understand the trance-methyl processes, research is being conducted into a compound group of various vegetal origins influencing the natural defensive system of the plants, some members of which have been proven to possibly play a role in human prevention as well.

Published results of different approaches have proved that among several vegetal bioactive molecules the betain (beetroot is an important source of it), and the resveratrol (red wine is one of its important sources) have characteristics blocking free radicals, and an antibiotic effect in charcinogenesis, reducing oxidative stress. The goal of our measurements, based on short term experiments with rats, was the detection of the changes resulting from the exogenic enlargement of mobilizable methyl-groups.

Our measurements were aimed at inherences that could enhance our understanding of the changes due to the quantitative enlargement of the methyl-pool.

During the experiments, male Wistar rats (5 animals per group) were treated for ten days. The control-group was fed with rat-nutriment only. The normal- nutriment -fed groups, treated with red wine and alcohol, received a daily amount of 8 ml per kg of body weight of a10,5 % alcoholic solution. The fat-rich nutriment -fed groups received cholesterol (2%), sunflower-seeds oil (20%) and cholic-acid (0,5%) mixed into the nutriment. The groups consuming beetroot too, received 2 gramm/kg of body weight lyophilized beetroot-powder mixed into the usual nutriment or into the fat-rich nutriment.

At the end of the treatment, we measured, besides the routine- laboratory parameters and the redox- parameters, the methylation- rate in the samples of blood and homogenized liver. We defined the bound endogenous HCHO with dimedone as adduct- forming compound as formaldehide.

We used pre-experiments to adapt the method earlier used for the examination of phytogenous tissues to the planned experiments. After that, we optimized the appropriate model- preparation and the enactable quantitative proportions to the reproducible detection. Finally, we carried out the measurements using the method of thin-layer chromatography, which enables, with the application of the appropriate standard, the simultaneous qualitative and quantitative analysis or comparison of 10 or 12 sample isochrones on a thin film-slab. Further advantages of the method are relatively simple model-preparation and quick and efficient separation.

Comparative study of oxidative stress parameters in critically ill patients

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Free radical reactions play an important role in the pathohysiological changes in critically ill patients, but there are only few data available regarding to the dynamism of oxidative stress during treatment of critically ill patients. The purpose of this study was to follow and to compare the time course of oxidative stress during treatment of ICU patients.

Patients with burn injury (n=26), sepsis (n=14), polytrauma (PT) (n=13), and acute lung injury (ALI) (n=22) were involved in the study. Blood samples were taken from patient on admission, and on the following 3-5 days. Concentration of malondialdehyde (MDA), reduced gluthation (GSH), protein sulfhydril (PSH) groups, the activities of superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO) enzymes were measured spectrophotometrically. Production of reactive oxygen species (ROS) in whole blood was measured by luminol dependent chemiluminescence following phorbol-myristate-acetate stimulation. Blood samples from healthy volunteers (n=9) served as the control.

While the white blood cell count significantly decreased in burned patients during the treatment, it remained on high level in the other groups. Marked granulocytosis and lymphocytopenia was observed initially in all groups that started to normalize only in burned patients from the day 4. ROS production was significantly elevated in septic and ALI patients from admission, but in burned and PT patients it rose significantly from day 3. Plasma MDA level significantly exceeded the control values, peaking on the days 2 and 3 in all groups. Plasma MPO level was significantly elevated in burned, septic and ALI patients from admission, but in PT patients it rose significantly from day 4. PSH level was significantly reduced in septic patients from admission, and in burned and PT patients from the day 2 and 3. GSH level significantly decreased in burned, PT and ALI patients from the day 2 and 3, while in septic patients it stagnated on a low level during the observation period. SOD enzyme activity was below the level of healthy population in most of the patients group, while catalase enzyme activity significantly exceeded it in all groups.

Significantly elevated levels of pro-oxidant markers with parallel decrease in endogenous antioxidants confirmed the presence of marked oxidative stress in critically ill patients. Time course of changes in oxidative stress parameters diverged markedly in critically ill patients mirroring the pathohysiological changes in different diseases. The significant differences in some oxidative stress parameters in survivor and non-survivor patients may have prognostic value.

The work was supported by the OTKA K060227 grant.

Fatty acid composition of human milk in Hungary with special attention to trans fatty acids

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Survey of fatty acid composition of 66 human milks obtained from 19 counties and Budapest, Hungary was performed in a research project supported by the Scientific Council for Health, Hungary. The selection of pregnant women was met all the requirements of WHO/GEMS Food representative sampling protocol, which was performed by the National Institute for Food and Nutrition Science (NIFNS) with the cooperation of health visitors on the basis of several hundred questionnaires. Human milk samples were collected from mothers below 30 years having first partum and had been living for 5 years in the same place. Country representative samples were collected within 2-8 weeks following the delivery with the help of health visitors and were transported to laboratory of NIFNS. The analysis of fatty acid composition, included trans-fatty acids was performed by gas chromatography.

In Hungary, this kind of monitoring was the first one. The total fat content of the samples was in the range of 0.3-6.2 g/100 g. 10 samples were close to the average fat content (4 g/100g) published in the Hungarian food composition table. Fifty-four samples had lower while two samples had higher fat content. The range of saturated fatty acids (SFA) (C8:0, C10:0, C12:0,

C14:0, C16:0, C17:0, C18:0) in total fatty acids was between 37.5-78.3%. In eleven counties six samples had 2-16% more saturated fatty acids than the average 44% published in Hungarian food composition table.

The percentage of the monounsaturated fatty acids (MUFA) as C14:1, C16:1, and C18:1 was in the range of 13.2 and 43.5. The ratio of polyunsaturated fatty acids (PUFA) including C18:2n-6, C18:3n-3, C18:3n-6, C20:3n-6, and C20:4n-6 was between 6.9 and 25.5%. The level of essential linoleic acid showed a very wide range as 6.9 and 23.7% in total fatty acids while the ratio of linolenic acid was in the range of 0-1.3%.

Among the trans fatty acids (TFA) elaidic acid (C18:1n-9t) originated from the hydrogenated vegetable oils and the linoleic-acid isomers as C18:2t9,t12, C18:2c9,t12, C18:2t9,c12 of ruminant origin could be identified. The ratio of elaidic acid in total fatty acids was 0.07-5.04% that means 0-150 mg elaidic acid in 100 g milk fat calculated on the base on fat content. In one sample 174 mg elaidic acid in 100 g milk was measured. The ratio of C18:2 isomers in total fatty acids was below 0.7%.

During the lactation, the fat composition of the human milk is highly influenced by the fatty acid composition of the diet. Data of this survey shows that the much higher level of SFA, the lower values of MUFA and PUFA, as well as the essential fatty acids in the human milk are due to unhealthy diet. The appearance of TFA in human milk is due to the consumption of foods containing hydrogenated vegetable oils one day before sample collecting. According to a national survey done by NIFNS TFAs present in many industrially produced foodstuffs in Hungary, as well. The TFAs have adverse physiological effects on the development of new-borns; these fatty acids can cause irreversible metabolic changes. The TFAs are able to inhibit the formation of long chain PUFAs as arachidonic and docosahexanoic acids which are inevitable during the brain development of the new-borns, as well as in the metabolic pathway of prostaglandins and thromboxans, the main responsible factors in balancing the blood viscosity and the formation of thrombus.

The research was supported by the Ministry of Health, Hungary (ETT 107/2006).

What do we know today about lycopene?

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Lycopene is an acyclic carotenoid molecule that does not take part in the synthesis of beta-carotene because of the lack of beta rings. Lycopene is a very powerful antioxidant *in vitro* and *in vivo*, as well. Lycopene shows a marked preventive effect against certain cancer and cardiovascular diseases partly thanking to its antioxidant characteristics. Main dietary sources of lycopene are tomato and foods prepared with tomato, watermelon, red grapefruit and some other exotic fruits. Scientific observations have proved that agricultural practices and food industrial processes significantly determine the lycopene content of fresh or prepared foods. In the frame of a 10 years' cooperation with Szent István University Department of Horticultural Technology the National Institute for Food and Nutrition Science (NIFNS) have measured the lycopene content of at least two dozen tomato varieties, and investigated the effects of horticultural techniques and weather conditions on lycopene level of tomato fruits. It was also studied how food industrial processes and dish preparing techniques determine the lycopene level of food products, finally a functional food with increased lycopene level was prepared with the use of by-products of tomato industry. Dietary lycopene intake was estimated in two small groups of Hungarian population and based on the representative nutrition survey done by NIFNS in 2003-2004, a population based intake was also calculated.

It was proved that lycopene accumulates in the tomato fruit during ripening, the correlation between the colour index and lycopene content can be drawn by a second order equation. Since the optimal temperature for lycopene synthesis is between 16-21°C, significantly lower level of lycopene by 25-30% could be detected in fruits directly exposed to sunlight having higher surface temperature than in that being in the shadow of leaves and having lower surface temperature. Significantly different lycopene levels were observed in different tomato varieties, the highest level (9,55-13,4 mg/100 g) was observed in industrial cultivars, middle values were in fruits of eating varieties harvested in green house (7,0-8,3 mg/100 g), while the lowest levels (4,90-8,02 mg/100 g) could be detected in tomato cultivars for fresh consumption harvested in open air. It was established that several factors including harvesting date or more punctually the weather conditions 5-10 days before the harvesting, the water-stress, the increased CO_2 level, and the grafting significantly modify the lycopene level of berries. Based on the consumption data the lycopene intake was estimated in a children's (n=502) and an adult's (n=205) group as 2.98±4.71 mg/capita/day, and 4.24±8.47 mg/capita/day, respectively. Data showed very big differences among subjects. Using the data

of the representative nutritional survey the population intake was around 2 mg/capita/day, but the lowest and highest levels showed very wide range of intake (0-40 mg/capita/day).

Lycopene has an excellent antioxidant capacity, its preventive and health-promoting properties are well-known and widely proved epidemiologically and experimentally, as well. Climate conditions in Hungary make possible to produce very valuable tomato fruits either economically or nutritionally. Increased consumption of fresh tomato and tomato-based foodstuffs can play an important role in the risk reduction of non-communicable diseases which are in connection of diet and especially increased free radical reactions.

Our preliminary data during the investigation of injuries following suprarenal aortic clamping in rats

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During various surgical interventions (for example: organ transplantations, vascular surgery, tumor removal) it is often necessary to clamp aorta and/or greater arteries for shorter or longer period of time, which disturbs the blood supply of organs/regions and during the following reperfusion as postoperative complications, further injuries can occur. As its most serious complication, systemic inflammatory response syndrome or as part of the syndrome, multiple organ failure can evolve. In our experiments, we aimed to investigate the development of the triggering factors of this condition, and to set up a suitable, appropriate experimental model for also studying the protection of this condition.

Adapting C. J. Shields et al. (2003) aorta occlusion model with small modification, a 30-minute ischemia followed by a 120-minute reperfusion was examined in male Wistar rats. We used 60mg/kgbw of thiopental for anesthesia. After cannulation of femoral artery, blood samples (0.5 ml per each) were taken before ischemia, prior to clip removal, and at the 1^{st}-20^{th}-60^{th}-120^{th} minutes of the reperfusion. The blood-gas analysis and the hematological parameters were immediately measured, and to determine the liver enzymes' levels we stored plasma samples on -70 C° degree until usage. The controls were sham-operated animals.

According to the blood-gas analysis, the pH levels remained within physiological range in both groups (pH = 7,35 – 7,45), the arterial pCO_2 and pO_2 values presented small changes during the experiments. Within hematological parameters the amount of white blood cells significantly increased in the I/R groups compared to controls and the extent of ascent was 50% at the end of the reperfusion. Some important parameters of red blood cells showed slight changes. The liver enzymes, especially the GOT levels increased with 67% towards the end of the reperfusion and the GOT/GPT proportion raised in the I/R groups as well.

In our in situ rat model, according to the examined parameters after I/R systemic inflammation, microcirculatory problems of some organs and results showing hypoperfusion damages were obtained. To determine the multiple organ failure and also for further standardization, we are continuing our researches.

Grants: OTKA K-67779 (2007-2010)

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Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Antioxidant property of *Grindelia robusta* infusum in the function of steeping time

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Gumplant (*Grindelia robusta* Nutt.) is a perennial plant growing wild in California, cultivated in Europe. *Grindelia robusta* has been found to be especially efficient in spasmodic asthma, giving prompt relief, and cures effectively in cases previously rebellious to medication. Its expectorant effect is also remarkable. Since in the indication fields in which the gumplant used the role of free radicals is proven, the aim of the work was to study the antioxidant properties of plant.

Gumplant was collected from Transylvania, from Botanical Garden of University of Medicine in 2007, Tirgu Mures, Romania. Aquous extracts were made from different parts of the plant (flower, stem and herba) by infusing for different time (5, 10, 30, 60, 120 min). Total scavenger capacity in extracts was determined by a chemiluminescence method. Hydrogen donor ability and reducing power were measured by spectrometric methods.

Hydrogen donor ability and reducing power vary considerably in the function of the steeping time and the concentration applied. The best results were obtained for concentrated extracts in all cases. Hydrogen donor ability and reducing power of teas generally increased with the increasing steeping time. Total scavenger capacity of flower extract also changed similarly, while significant total scavenger capacity of stem and herba was measured in extracts obtained by 5 min steeping time. In summarizing, the highest antioxidant values were obtained after 120 min steeping time in the case of flower extracts, while the optimal(best) steeping time in case of stem and herba extracts vary in large scale of time depending on the kind of antioxidant measurement.

The antioxidant properties of Grindelia robusta extracts depend on several factors, as plant parts, extraction procedure and concentration. In general 30-120 min steeping time proved to gave the highest antioxidant values except for that 5 min steeping time is enough for relevant total scavenger capacity of steam and herba extracts.

Prooxidant effect of trichothecene mycotoxins in poultry

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Fusarium moulds present is most of the temperate climate areas of the world and those are produce trichothecene mycotoxins, such as T-2 toxin, HT-2 toxin, scirpentriol, nivalenol, diacetoxyscirpenol and deoxynivalenol. There are numerous data that trichothecene myco-toxins affect the antioxidant status of animals, primarily due to their pro-oxidant effect. However, not clear whether the pro-oxidant characteristic of these mycotoxins is a direct or indirect effect. Chemical structure of trichothecenes, presence of epoxy group in the trichothe-cene ring, supports the direct effect through their metabolism by the xenobiotic transforming enzyme system.

The objective of our series of studies was to evaluate dose- and time-related effects of the most important trichothecene mycotoxin, T-2 toxin, on glutathione redox and lipid peroxide status of chickens. The birds were fed with diets experimentally contaminated with different doses of T-2 toxin (0.12, 0.4, 1.5, 2.05 or 2.35 mg kg^{-1}) without or with antioxidant supplementation (vitamin E: 10.5 mg + selenium 0.045 mg $animal^{-1}$ day^{-1}) in short-term (14 days) or long-term (39 days) studies. In each experiment five animals were exterminated from each group at days 3, 7 and 14 in short-term and at days 21 and 39 in long-term trials. Blood and liver samples were taken, in which reduced glutathione (GSH), malondialdehyde (MDA) concentration and glutathione-peroxidase (GSHPx) activity were measured.

The results showed that there were not dose-related changes in the parameters investigated, however long-term effect of T-2 toxin was found, mainly in liver. According to the changes of the different parameters in different tissues it can be stated that liver showed the most marked changes which followed by blood plasma and red blood haemolysates. Antioxidant

supplementation of the experimentally T-2 contaminated diet resulted improvement of the antioxidant and moderately in lipid peroxide status.

The possible causes of the lack of dose-relation would be the environmental factors, e.g. temperature and light regimen, also partially different genetic background of the experimental animals, even all of them was the same hybrid. The other possible cause would be the presence or lack of natural metabolites of T-2 toxin or some other not-identified trichothecene mycotoxins, because the experimental contamination was carried out using crude extract from the mainly T-2 toxin producing moulds, *Fusarium sporotrichioides* or *Fusarium tricintum.*

In conclusion it can be stated that T-2 toxin exposure has some pro-oxidant effect also activated or impaired the amount/activity of the glutathione redox system but its effect depends on the duration of the study also some other factors. Additionally, the question, that T-2 toxin has direct or indirect pro-oxidant effect remains open.

Effects of postconditioning on kidney ischemia/reperfusion injury in hypercholesterolemic rats

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Ischemia/reperfusion injury frequently threats the integrity of the organs during surgery. The protective effect of postconditioning (PK), the short repetitive ischemia/reperfusion cycles, applied in the beginning of reperfusion, has been improved the outcome in vital organs. Signaling cascades are induced by PK interfere in several points to preconditioning, which is blocked by metabolic diseases, such as insulin resistance and type 2 diabetes.

The aim of our study was to compare the efficacy of PK after reperfusion injury of both kidneys in metabolically healthy and hypercholesterolemic rats.

Male Wistar rats (N=30) were divided into two groups. Control group of the animals were fed by normal rat chow, the treated group (n=18) was fed with 1.5% cholesterol containing diet for 8 weeks. Both groups of rats were divided to further two subgroups, and were anaesthetized by ketamin: diazepam. One subgroup of rats was subjected to 45 min ischemia and 2 hours reperfusion, in the other subgroups 4x5 min ischemia/reperfusion cycles were applied in the early phase of reperfusion. After 2 hours of reperfusion blood and tissue (kidney, heart, liver, lung) samples were taken. Serum cholesterol, glucose and triglyceride levels were determined by photometric methods. Kidney function was characterized by serum urea, and creatinin levels. Inflammation and oxidative stress were characterized by the measurement of TNF-α and oxLDL concentrations (ELISA) and PMA induced free radical production capacity of whole blood by chemiluminometric method. Tissue injury in kidney was determined by formaline-fixed, paraffin embedded tissue sections (5 μm), stained with PAS and HE. TNF-α levels were also determined by immunohystochemistry.

Serum cholesterol and triglyceride levels were significantly higher in cholesterol fed rats than in control ones. Serum urea and creatinine levels were same in control and hypercholesterolemic groups. A significant elevation was observed in TNF-α level ($p<0.01$), PMA-induced free radical production ($p<0.05$), and in lipid peroxydation (oxLDL; $p<0.05$) after I/R injury in healthy rats, which reduced almost to the normal levels in PK ones. In hypercholesterolemic rats neither the elevation, nor the postconditioning induced reduction were not as significant as in the healthy rats. Surgical intervention caused a great elevation in serum glucose and insulin levels ($p<0.01$). PK caused a further elevation in insulin levels, while the TNF-α concentration and free radical levels were reduced. Tissue TNF-α level, measured in hypoxia sensitive papilla, was significantly higher in cholesterol fed animals, than in control rats, and this high level was not able to change in response to PK. In healthy animals PK caused a significant reduction in tissue TNF-α level, as well.

PK proved to be a very effective defense against I/R in healthy animals, but it was ineffective in hypercholesterolemic ones.

Volume 53(Suppl.1) 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Serum selenium concentrations of gestational diabetic and control pregnant women in the second trimester of pregnancy

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High serum selenium concentrations are positively associated with the prevalence of type 2 diabetes according to recently published data, whereas in an intervention study, patients who received 200 µg selenium per day as oral supplementation for seven years, had a significantly higher risk of developing type 2 diabetes mellitus compared to the control group. In a previous study, serum selenium levels in gestational diabetic pregnant women were slightly, but significantly higher than in control pregnant women. This finding needed to be confirmed by a larger number of study participants.

To determine serum selenium concentrations and plasma total glutathione peroxidase activity of 31 gestational diabetic and 20 control pregnant women between the 24th and 28th week of pregnancy.

Serum selenium concentrations were measured by hydride generation atomic absorption spectrometry. Plasma glutathione peroxidase activity was determined by an end-point direct assay in the presence of reduced glutathione and cumene-hydroperoxide as co-substrates. Statistical analysis was performed using the Microsoft Excel 7.0 and Statistica™ 4.0 software packages.

Serum selenium concentrations were significantly higher in gestational diabetic (50.4±14.4µg/l) compared to control pregnant women (41.1±7.7µg/l, p=0.004). Plasma total glutathione peroxidase activity did not differ between the two groups of pregnant women (3.30±0.95 E/g protein in case of gestational diabetic and 2.84±0.60 E/g protein in case of control pregnant women). Serum selenium concentrations correlated significantly with plasma glutathione peroxidase activity in control pregnant women. In gestational diabetic study participants serum selenium concentrations correlated inversely with fasting plasma glucose values (p=-0.80).

This study confirmed our previous finding of significantly higher serum selenium concentrations in gestational diabetic compared to control pregnant women. The reasons for this observation are unclear; however, the correlation value shows that serum selenium levels seem to either influence or be influenced by fasting plasma glucose concentrations. Despite higher serum selenium levels in gestational diabetics, selenium-dependent glutathione peroxidase activities are similar in both groups of pregnant women.

Examination of microvascular reactivity in juvenile essential hypertension and haemodialysis

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The relationship of juvenile essential hypertension and impaired microvascular function has not yet been demonstrated. In contrast with hypertension in adulthood, a simultaneous assessment of the markers of oxidative stress and the microvascular reactivity has yet not been performed in adolescent patients with essential hypertension.

To compare the microvascular reactivity and markers of oxidative stress in overweight and lean hypertensive adolescents (OHT, LHT), and young haemodialyzed (HD) patients as positive controls.

Twenty-three OHT adolescents, 10 LHT adolescents, 12 young HD patients and 19 controls were enrolled. Microvascular reactivity of the forearm was assessed by means of laser Doppler flowmetry, measuring alterations of the blood perfusion of the microvasculature. Endothelium-dependent and -independent vasodilation, informative of the endothelium and smooth muscle layers of the vessels, were assessed by means of acetylcholine and sodium nitroprusside iontophoresis, respectively.

Maximal vasodilation was achieved by local heating of the skin to 44°C. The obtained perfusion values were expressed as relative to the basal values. We also determined the ratio of the whole blood oxidized/reduced glutathione (GSSG/GSH), the erythrocyte malondialdehyde levels and the activities of erythrocyte antioxidant enzymes.

Microvascular reactivities in both tests were moderately decreased in the two hypertensive groups, and significantly impaired in the HD group, as compared with healthy controls. Ratios GSSG/GSH were increased in all patient groups, being highest in the HD patients. The erythrocyte malondialdehyde levels and the activities of superoxide dismutase and glutathione peroxidase were significantly elevated in the HD group.

Our results suggest that the impairment of the microvascular reactivity does not precede the development of juvenile essential hypertension: an imparied microvascular reactivity is more likely a consequence and not a cause, being related to the degree of oxidative stress.

Improvement of beer's flavour stability by adding antioxidant vitamins

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Thanks to the growing tendency of the consumer's demands, it is important to find possibilities to improve the shelf life of different foods. The favour stability of a food is an important part of its quality. The flavour changes in beer could be caused by the formation of radicals resulting from the ingress of oxygen.

Vitamin E and C are widely regarded as important dietary antioxidants. These vitamins were added to beer samples at different technological stages using a concentration range between 0 and 40 mg/L for Vitamin C and from 0 to 4 mg/L for Vitamin E. The aim of this study was to determine the flavour stability of these vitamin enriched samples.

The flavour stability of beers can be determined by Electron Spin Resonance (ESR). One of the examined parameter is the lag time. This parameter was determined at packaged sample technological stage in each case. It is in direct connection with the quantity of natural antioxidants found in beer. The formation of hydroxyl radicals will start only after this period of time. The ESR method can be used to analyse the effect of antioxidant vitamins such as vitamin E and C on the flavour stability of beer.

Comparing the different technological stages for vitamin addition, it can be established that the best lag time values were measured when the vitamins were added to the wort after cooling. If vitamin E concentration was higher than 4 mg/L at original pH or vitamin C concentration was higher than 30 mg/L at lower pH, the lag time was higher than 100 minutes. Vitamin addition at the end of fermentation increases the lag time in some cases, but adding vitamin is not recommended in the case of packaged beer.

In the case of original pH ascorbic acid always had a smaller effect on the value of lag time than vitamin E. If these vitamins were added together, their effects were combined so the presence of ascorbic acid reduces the effect of vitamin E.

Conclusions: On the grounds of these facts it can be stated that if the growth of the lag time is the target, individual vitamin addition is the right way to do it. The best results were received when only vitamin E was added to the wort at original pH, or when vitamin C was added to the wort samples separately at a lower pH value.

Volume 53(Suppl.1) 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Detection of oxidative stress from bronchoalveolar lavage fluid in animal model

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Oxidative stress results from an oxidant/antioxidant imbalance in favour of oxidants. A large number of studies have demonstrated that increased oxidative stress occurs in airways diseases, but there is now substantial evidence that it really plays an important role in the injurious and inflammatory responses in acut lung injury and in asthma.We developed a model that allows the quantification of the airway responsiveness (BHR) and the characterizations of the BAL cellular profile repeatedly within the same rat. The detection of carbonyl proteins (CP) is a wide spread method to detect oxidative stress, where the values are increased. In the present study with the help of our method we examined, whether oxidative stress measured by CP plays a role in the patomechanism of different lung abnormalities, so acut lung injury and asthma in animal model.

We studied 28 male white, Wistar rats (weight range 350–500 g). The animals were housed in a healthy colony and allowed food and water ad libitum. Anesthesia was induced, intubations were performed, muscle relaxation was achieved and mechanical ventilation was used, BALF was collected. The BAL fluid was then centrifuged and CP was detected by spectrophotometry method .Acute lung injury was caused by intraperitoneal (ip) injection of E Coli lipopolisacharide (LPS) and asthma was induced by the combination of ip. and inhalative ovalbumin (OVA).

The acute lung injury caused by LPS was due together with significant oxidative stress which was reflected by the increase of the CP values in BALF. OVA sensitization was connected with a less significant oxidative stress right after the procedure, but the CP values increased further until the next measurements, one month later (12,9 vs 13,9 vs 16,4).

The results confirm the role of oxidative stress in the patomechanism of acute lung injury and asthma in rats. Our method is suitable to detect the oxidative stress in animal model and will be also suitable later to investigate the protective effects of different antioxidants substances.

Examination of biochemical parameters of oxidative stress in childhood asthma

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Increased levels of reactive oxygen intermediates (ROI) and cellular injury have been implicated in many pulmonary diseases including COPD, cystic fibrosis and asthma bronchiale. According to earlier examinations, the production of ROI was elevated in the asthmatic airways due to the action of macrophages, eosinophils and neutrophils, which led to bronchial hyperresponsiveness and caused more severe airway inflammation. ROI also play important roles in the modification of immune response and in the structural alterations of the lung parenchyma.

The aim of our study was to analyse the alterations of several biochemical parameters such as malondialdehyde (MDA), carbonylated proteins, ratio of oxidized/reduced glutathione, vitamin E concentration, total antioxidant capacity and activities of antioxidant enzymes (catalase, glutathione reductase, glutathione peroxidase, superoxide dismutase). The blood samples were obtained from asthmatic children (n=21) and healthy controls (n=12). The clinical state of the patients was compared to the examined biochemical markers.

According to our results, elevated oxidative stress was observed in asthmatic patients even with stable clinical condition. Elevated MDA (mean. 0,86 vs. 0,58 nM/mg prot.) and carbonylated proteins (mean. $4{,}711 \times 10^{-2}$ vs. $3{,}859 \times 10^{-2}$ µg/mg prot.) levels were detected from the blood samples of the patients versus controls.

Our results also proved the presence and importance of oxidative stress in asthma and the possible use of biochemical parameters in the clinical practice. Additionally we set out to examine the expression of the gp91phox subunit of NADPH oxidase enzyme and HO-1 enzyme from blood.

Genotypic, seasonal and maturity stage variability in antioxidant capacity of stone fruits

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In Hungary, nutrition-related diseases (heart and vascular attacks, different types of cancer) are among the main causes of mortality. Several epidemiological studies suggested that consumption of fruits and vegetables can help in the prevention of these degenerative diseases. However, the statistics show that Hungarian people do not eat enough fruits and vegetables. One of the possible solutions would be the consumption of fruits with enhanced functional properties and higher levels of the required bioactive compounds. Although berry fruits are generally considered to contain outstanding levels of antioxidants, stone fruits are less known from this aspect. The aim of our examinations was to characterize the antioxidant capacity of stone fruits and to clarify the influencing effect of the genotype, ripening status and cultivation plot. In addition, we wanted to assess how the anthocyanin and vitamin C contents contribute to the antioxidant capacity of sour cherries.

In the present study, the antioxidant capacity was measured with ferric reducing ability of plasma (FRAP) and a photochemiluminescence method (ACW) in 11 sour cherry, 19 sweet cherry, 20 Japanese plum, 6 cherry plum and 6 apricot cultivars. In addition, the content of total phenolics (TPC), carbohydrate, vitamin C, monomeric anthocyanins and nutrient elements were also determined.

Cultivar averaged mean values of FRAP and TPC results were the highest in sour cherry, and the lowest in cherry plum. Variations between species were the highest in case of sour cherry and sweet cherry. Most of the sour cherries reached the FRAP values of raspberries, which characteristically contain high antioxidant capacity (5-6 mmol AS/L). A sour cherry cultivar reached the outstanding water-soluble antioxidant capacity value of blackberries and elderberries. This attracts attention to the alluring perspectives of this genotype. Correlations between the FRAP and ACW values were close ($r = 0,78$). In average, the sour cherry cultivars contained the highest amounts from several nutrient elements (e.g. Al, Cu, Fe, Mn etc.). The lowest element quantities were detected for Japanese plum cultivars. Levels of Al and K were outstanding in cherry plums. In case of some neurodegenerative diseases, patients should eat such fruits with lower contents of redox active metals (e.g. Japanese plums), because these patients should avoid these metals. We measured the glucose and fructose contents of sour cherries. The highest values of these two monosaccharides were detected in 'Cigány C404' and in 'Cigány 59' cultivars, while VN-07 contained the lowest levels from these sugars. The highest anthocyanin values were observed in fruits of cultivar candidates. Our analysis revealed a small difference between the lowest and highest vitamin C contents. Genetic background of cultivars forms the decisive factor in determining fruits' antioxidant capacity, although the cultivation plot and season may have also considerable modifying effects. Based on our results we can conclude that functional food products can be established from stone fruits.

This work was financed by the NKTH-OTKA K68921 grant.

Possible role of reactive oxygen species in the development of immunological tolerance

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Since plasmacytoid dendritic cells (pDCs) are professional antigen-presenting cells, they have an important role in the polarization of the adaptive immune responses toward inflammation or antibody production. As professional interferon type I producing cells, the pDCs also possess a significant antiviral function. Previously, the pDCs were thought to be found only in bone marrow, lymphoid organs and blood; however, recent studies have indicated that they can also be detected in inflamed tissues. The pDCs leaving blood circulation and entering peripheral tissues are affected by - in addition to many other factors - the reactive oxygen species produced by inflammatory reactions. The effects of oxidative stress on the functions of pDCs have not been examined yet.

Our goal was to investigate how oxidative stress can influence the viability, phenotypic characteristics and cytokine production of non-activated pDCs or those activated by Toll-like receptor 9 (TLR9) agonists. We also studied how the experimental oxidative stress conditions, which were used for pDCs, change the viability of other lymphoid and myeloid cells.

Cells were isolated from peripheral blood of healthy donors by the method of magnetic separation. The xanthine oxidase/xanthine (enzyme-substrate) system and hydrogen peroxide were used to create the conditions of oxidative stress. After treatment, the alterations in viability and the phenotypic changes of cells were detected by four-color flow cytometry. The levels of IFN-ĐIL-6 and TNF-α cytokines were measured in the supernatants of cell cultures by ELISA.

Our data demonstrate that pDCs are very sensitive to oxidative stress, because exposure to reactive oxygen species significantly decreases their viability, lowers the expression of all examined surface antigens (BDCA-2, HLA-DQ, BDCA-4) and reduces their cytokine production. Our results also indicate that oxidative stress eliminates the activating effects of TLR9 agonists on pDCs. We found that there are significant differences in the sensitivity of lymphoid and myeloid cells to oxidative stress. The lymphoid cells, similarly to pDCs, showed strong responses to oxidative stress, whereas myeloid cells did not.

Lowered expression of cell surface molecules and decreased cytokine production suggest that pDCs exposed to reactive oxygen species produced by inflammatory cells may induce immunological tolerance instead of adaptive immune response upon interaction with naive T-cells. This phenomenon may provide an opportunity for a new, dendritic cell based therapy, in which pDCs treated with reactive oxygen species *in vitro* can be used to create immunological tolerance to a certain antigen *in vivo*, for example in the treatment of autoimmune diseases or severe allergic inflammations.

Measurement of redox parameters in the blood plasma of dogs with renal disease

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Renal disease is common in older dogs and cats, which can lead to cirrhosis and kidney failure. The symptoms appear several months after the onset of the disease when about 66-75% of the kidney tissue is irreversibly damaged. Many old dogs carry some degree of kidney disease, but its progress can be slowed down with an appropriate treatment. Correct diagnosis and therapy require the knowledge of the mechanism of the development of kidney disease and of the parameters, which may play important role during an effective treatment. In addition to previously described medical parameters, little is known about the molecules leading to oxidative stress in canine kidney failure.

Based on literature data, find redox parameters that can be useful during the treatment of kidney diseases. Methods: Blood samples of 60 healthy and 81 dogs with kidney disease (blood plasma creatinine concentration >140 μmol/L) were used to determine different redox and routinely measured laboratory parameters. Whole blood stationary free radical concentration was determined using electron spin resonance (ESR) spectroscopy. Malondialdehyde and hydroxynonenal were measured as markers of lipid peroxidation, while protein oxidation was assessed by production of carbonylated proteins. Some antioxidant parameters relevant in kidney disease were also determined: glutathione ratio, enzymatic activity of SOD, as well as FRAP (ferric reducing ability of plasma) and TAS (total antioxidant status).

Free radical concentration of whole blood was significantly higher in samples taken from dogs with kidney disease compared to those taken from healthy animals. Malondialdehyde on its own showed no differences between the two groups, only when measured together with hydroxynonenal, a significant raise in lipid peroxidation was observed in renal disease. Plasma protein carbonylation was significantly higher in the group presenting kidney disease. Within the measured antioxidants reduced glutathione showed differences between the two groups as its levels were higher in diseased dogs compared to their healthy counterparts, and the activity of SOD increased in the same samples as well.

Concentration of free radicals in the blood of dogs with kidney disease is higher than in healthy animals. Lipid peroxidation increased in blood plasma of dogs with kidney disease. Levels of protein carbonyls also increased in blood plasma of dogs with kidney ailment. An induction of the antioxidant mechanism was seen in the blood plasma of sick dogs.

Markers of oxidative stress could be observed in the blood samples of dogs with kidney disease. Question: in addition to an antioxidant rich diet, what other recommendations can be made to slow down the progression of kidney failure and to allow dogs to live as close to normal life as possible under the given circumstances?

This work has been supported by grants of the Hungarian Research Foundation OTKA 61566 and 49753.

Preventive effect vanadium, zinc and bioflavonoids on the onset of diabetes in BB rats

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Vanadium, other trace elements and bioflavonoids have been shown to be beneficial in the treatment of animal models of type 1 and type 2 diabetes. The aim of the study was to evaluate the preventive effects of vanadate (as ammonium metavanadate), zinc chloride and bioflavonoids in prediabetic BB rats.

80 BB rats were divided into 4 equal groups. Group "V" was treated with ammonium metavanadate (0.1 mmol/l), "Z" with zinc chloride (0.1 mmol/l), and "BF" with Flavin 7® (nutrition additive with bioflavonoids, 0,2 mg/l) in drinking water from 21st day after birth to 171st day of their life, and compared with "C" – control group on pure tap water. In each group food and water intake, urine output and body mass were followed regularly The manifestation of diabetic state was monitored through blood glucose, glycosuria and glycosylated hemoglobin determinations. Antioxidant system activity was estimated through enzyme (red cell superoxide dismutase, red cell catalase, whole blood glutathione peroxidase) as well as total antioxidant status and glutathione assays.

The age of onset of diabetes and its incidence were significantly higher in "BF" and "V" groups as compared to controls ($p<0.001$), and zinc treated group ($p>0.05$). In overtly diabetic rats blood glucose was higher in control group than in "V" and "BF" groups, $p<0.001$. Decrease of parameters of the antioxidant status, at the onset of the treatment as well as immediately after its cessation showed a drop in the treatment groups, but later increased slowly and continuously until the end of the experiment. The activity of antioxidant enzymes increased slowly from the beginning of study up to the point of diabetes manifestation and decreased thereafter. The decline was less evident in rats treated with bioflavonoids.

Both bioflavonoids and vanadate delay the development and lower the manifestation rate of diabetes in BB rats which is not the case in zinc treated animals. The same compounds decrease hyperglycaemia in diabetic rats. Bioflavonoid supplementation could have a beneficial effect on antioxidant status in diabetes mellitus.

Effect of immunonutrition with omega-3 fatty acids on oxidative stress response in polytraumatized patients – Pilot-study

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A state of increased oxidative stress has been recognised in polytraumatic injury that was influenced beneficially by omega-3 fatty acids substitution in patients with type 2 diabetes mellitus. Moreover previous studies have shown that administration of omega-3 fatty acids mixed with other antioxidant substances resulted shorter postoperative periods in the intensive care unit.

We evaluate the effect of nutrition with omega-3 fatty acid on the polytraumatic injury induced oxidative stress.

13 patients were randomised to Intralipid and Omegawen groups, based on their parenteral feeding. There was difference only in omega-3 supplementation between nutrition of the two groups. Blood samples were taken on admission and during the following 5 days. We measured the level of malondialdehid (MDA), glutathion (GSH), plasma SH groups (PSH), the activity of superoxid dismutase (SOD), catalase (KAT), and peroxidase (MPO) enzymes, and the stimulated reactive oxygen species (ROS) production of whole blood. Injury Severity Score (ISS) and Simplified Acute Physiology Score (SAPSII) were calculated on admission. Clinical data, Sequential Organ Failure Assessment Score (SOFA), Multiple Organ Dysfunction Score (MODS) were calculated every day. Primary endpoints were the duration of ICU stay and the number of mechanical ventilated days. For statistical analysis we used Mann-Whitney U test and two-way ANOVA test.

The two groups were similar initially in ISS, SAPS II, MODS, SOFA. The MDA level was significantly higher in both groups compared to the control healthy group ($p<0.05$). We observed an elevating tendency in MPO enzyme activity in both

patients groups that was significantly higher on the 6th day compared to the controls. The induction time of ROS production was longer in the Omegaven group during the examination period than in the control group, and it was significantly longer on the 5th day compared to the Intralipid group. We detected higher catalase activity in Omegaven and in Intralipid group as well, but this activity was significantly lower on the second day in the Omegaven group versus Intralipid group. GSH and PSH levels weren't influenced by the treatment of omega-3 fatty acids.

These data suggest, that polytraumatic injury causes considerable oxidative stress, on which omega-3 fatty acid supplementation has only a moderate effect.

The study was supported by OTKA K060227.

In vitro toxicity testing of PPI dendrimers

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Dendrimers are a new type of promising synthetic polymers characterized by a dendric branched spherical shape and a high density surface charge. The defined structure of these molecules has led to the interest in dendrimers as substrates for the attachment of antibodies or agents for applications in a number of different areas of biology and medicine. However, information on the mechanisms of dendrimer-induced cytotoxicity and a cell death is still limited. Therefore, it is necessary to undertake studies to determine biological properties of these compounds *in vitro*.

Thus, the aim of our investigation was to compare the effects of poly(propyleneimine) (PPI) dendrimers (PPI with 25% maltotriose units attached to the surface) on cultured human ovarian cancer cells (SK-OV-3) and Chinese hamster ovary cells (CHO). The cells were exposed to various concentrations of dendrimers (ranging from 1 to 300 μM). The toxicity of PPI dendrimers was studied immediately after the incubation with dendrimer (24 h) or 24 h after removing the dendrimer from the medium.

The cytotoxicity of dendrimers was studied by a MTT assay. The morphological features of apoptosis and necrosis were examined by Nomarski DIC combined with a confocal laser scanning microscope (CLSM). The level of reactive oxygen species (ROS) was evaluated with fluorescenct probe: dichlorofluorescein-diacetate (H_2DCFDA) by flow cytometry. Changes in mitochondrial membrane potential were determined using JC-1.

Our studies demonstrated that PPI dendrimers exerted multiple suppressive effects on cancer SK-OV-3 cells, including proliferation inhibition, induction of an apoptotic cell death and a collapse of mitochondrial membrane potential. Most importantly, these compounds were more cytotoxic to cancer cells than to normal CHO cells.

These findings will help to understand the mechanisms of PPI dendrimer cytotoxicity in normal and tumor cells and open the possibility to use them in clinical applications.

Project "Biological properties and biomedical applications of dendrimers" operated within the Foundation for Polish Science Team Programme co-financed by the EU European Regional Development Fund.

Bioactive compounds in *Alliums* from Vojvodina - antioxidants

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Toughout recorded history *Alliums* especially garlic and onion played rich diverse commercial, culinary, and mystic roles. Today garlic and onion are used for their flavour, aroma and taste, being prepared domestically or forming basic materials for a variety of food manufacturing processes. Onions were among the earliest vegetables to be processed, canned, dried and frozen. Many epidemiological studies have suggested that certain natural foods could prevent the development of different diseases. Garlic and onion are such natural foods. They have a variety of pharmacological effects including tumour cell growth inhibition and chemopreventive activity. Much of the data about human use came from reports of lowered rates and risks of disease (such as cancer) in people with relatively high levels of garlic or other *Alliums* consumption. People also use garlic

and onion to help with several different types of aliments, high cholesterol, high blood pressure, excess blood clotting and coagulation, atherosclerosis, inflammation, bacterial and fungal infections. Garlic is also functional food product composed of numerous macronutrients, vitamins, organosulfur active compounds.

The aim of our study was to investigate different cultivated (*Allium nutans* L., *A. fistulosum* L., *A. vineale* L., *A. pskemense* B. Fedtsch, *A. cepa* L. and *A. sativum* L.) and wild (*A. flavum* L., *A. sphaerocephalum* L., *A. atroviolaceum* Boiss, *A. schoenoprasum* L., *A. vineale* L., *A. ursinum* L., *A. scorodoprasum* L., *A. roseum* L. and *A. subhirsutum* L.), *Allium* species, in order to evaluate their antioxidant properties.

All the antioxidant enzyme activities were determined spectrophotometrically at 25°C using phosphate buffer (pH 7) plant extracts. The amount of reduced glutathione (GSH) was determined with Ellman reagent, lipid peroxidation (LP) was determined by the thiobarbituric acid (TBA) method. Hydroxyl radical (·OH) was determined by the inhibition of deoxyribose degradation, total flavonoids were estimated according to Marckam and soluble protein content was determined by the method of Bradford. Radical scavenging capacity was determined using 1, 1-diphenyl-2-pycril-hydrasil radical (DPPH) and ESR. Reduction of DPPH radical was determined measuring disappearance of DPPH. Total antioxidant capacity was estimated according to the FRAP. Lipofuscin pigments (LFS), were determined fluorimetricaly.

Our results are one more confirmation that antioxidant and scavenger activities influence the pharmacological activity of garlic and other *Alliums*. In leaves of *Allium fistulosum* L., LFS accumulation was also not observed. As LFS is generated as a product of tissue decay, caused by toxic oxygen species it means it has a high antioxidant capacity. The scavenger activity of *Allium fistulosum* L. was also high; in its presence, generation of the ·OH radical (the most toxic oxygen species) was reduced by 87.09%. Other results concerning *Allium fistulosum* L. support this assessment because the activities of all antioxidant enzymes SOD, CAT, GPX, and GP were high, concentrations of $O_2^{\cdot-}$, ·OH and MDA were low, and the quantity of GSH, flavonoids, vitamin C and soluble proteins were high, as was the carotenoids content.

Presented results indicated that crude extract of *Alliums* from Vojvodina exhibited antioxidant and scavenger abilities in all investigated plant parts especially in leaves. Therefore overground part of *Alliums* could be used as the source of natural antioxidants in the pharmaceutical, cosmetic and food industries for manufacturing antoxic products with potent medicinal and antioxidant activity.

Comparison of antioxidant power in fruits of commercial apple cultivars and cultivar candidates grown in Hungary

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The apple (*Malus domestica* Borkh.) has a privileged role among fruits in the temperate zone. Its consumption is not limited to any particular season because several cultivars are available to cover supply during the whole year. Furthermore, it can be processed, *i.a.* it is an essential components of baby foods. Its valuable inner contents greatly contribute to the wide applicability of this fruit. Apple has remarkable contents of energy and raw fibre and it is also a rich source of vitamins and mineral elements. Apple is one of the most consumed fruits in Hungary, and hence its valuable compounds (vitamins, minerals, polyphenolic compounds) may significantly contribute to the health-promoting effects of human diet.

The aim of this study was to characterize the inner contents, antioxidant power, total phenolic content and mineral nutrient element contents of commercial apple cultivars in comparison with perspective cultivar candidates and estimate their contribution to the coverage of physiological requirements.

Among the main inner content parameters, total phenolic content and antioxidant capacity (FRAP) were measured spectrophotometrically. Mineral element contents in fruits were determined by ICP-OES. Different apple genotypes (well-known commercial cultivars and perspective cultivar candidates) grown under the same conditions were used for the analyses. Antioxidants were compared in different parts (skin and flesh) of the apple samples.

Our results indicate significant differences in all measured parameters among the assayed cultivars and cultivar candidates. Different antioxidant assays revealed 2- to 3-fold differences between the lowest and the highest values in commercial cultivars and cultivar candidates. The antioxidant power of fruits was much influenced by the skin/flesh ratio as smaller fruits

with higher skin/fruit flesh ratios had increased antioxidant capacity compared with larger fruits. It indicates that the antioxidant compounds predominantly accumulate in fruit skin. Considering that all samples were collected in orchards located in the same region, these differences are likely to be explained by the different genetic backgrounds of cultivars and cultivar candidates. Some cultivar candidates were characterized by higher antioxidant capacities and mineral element contents than the main commercial cultivars pointing to the possibility for increasing health-benefits of apple even under constant level of fruit consumption.

This work was financed by the Jedlik Ányos programme NKFP-A2-2006-0084.

Temporal changes of antioxidant parameters in *Acorus calamus* L.

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Sweet flag (*Acorus calamus* L., Araceae) is widely used medicinal plant as extracts or dried rhizome for several diseases, for external or internal use, as well. Numerous studies performed its antioxidant effects such as decrease of lipid peroxidation in noise-stressed rat brain after application of alcoholic extracts of Acorus. Since, sweet flag is under protection in Hungary and we have relatively little information about antioxidant properties of Hungarian population we decided to estimate some antioxidant parameters and temporal changes of these during vegetation period.

Plant material was collected twice in 2008 (June and October) and after washing with distilled water leaves (L), rhizome with (H) and without bark (HL) were used freshly (homogenate) or as alcoholic and watery extracts made of dried drugs. Parameters measured were FRAP (ferric reducing–antioxidant power), glutathione (GSH) level and free radical scavenging ability using DPPH. Statistical analysis was performed using STATISTICA 8.0 software (analysis of variance and correlation).

Our results showed that homogenate and alcoholic extract of leaves had significantly higher FRAP-values compared to those of watery extracts, in June. Antioxidant capacity in rhizome was usually lower than in leaves. In temporal aspect, a significant decrease (40%) of FRAP appeared in alcoholic samples of leaves, while there were no changes in rhizome. Glutathione (GSH) level was 4-6-fold higher in leaves than in both forms of rhizome and was in significantly positive correlation with FRAP. Fraction of residual DPPH radical (%) was the highest in rhizome with bark (H) which means that it had quite low reducing ability, nevertheless, free radical scavenging capacity of homogenates of leaves and rhizome with bark showed to be significantly higher in October compared to June. According to FRAP we can make a sequence qualifying the three types of samples: homogenate > alcoholic extract > watery extract.

Our study was supported by OTKA T049503.

Table beet and red cabbage, as natural source of antioxidant compounds

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Free radicals derived from oxygen play an important part in the pathomechanism of different illnesses. Living organisms are supplies with an effective defence system against oxygen radicals. The first defence line is composed of antioxidant enzymes but different vitamins and low molecule compounds, such as phenols, thiols and flavonoids, are also effective against radicals. These compounds can be found in high quantities in vegetables. These compounds are mostly of polyphenol type and are able to bind free radicals and protect from the oxidation of biological molecules, membranes and tissues induced by active oxygen and free radicals. In evaluating bioactive content of vegetables an important role is provided to those compounds and are able to bind free radicals and protect from the oxidation of biological molecules, membranes and tissues induced by active oxygen and free radicals. Such are for example phenol type substances whose group includes pigment content as well. The colour materials of table beet and the red cabbage are suitable for natural pigment production and the same time they have favourable nutrition effect too.

During our experiment we measured FRAP and colour content in 4 red cabbage hybrids and 20 beet root varieties. In formation of bioactive substances of vegetables are very important the heritable quality parameters too. In this way we examined not only the different species, and the roil of varieties belong to them.

The red pigments were evaluated from diluted samples. A spectrophotometer was used to determine the absorbance of pigments: λ=538 nm for red pigments and λ=476 nm for yellow ones. For total phenol content the colour reaction to Folin-Denis reagents were evaluated at λ=760nm, by means of a katechin standard (mg katechin/100 ml). Total antioxidant content was expressed in the so-called FRAP values (ferric reducing ability of plasma) in μM/l. The method is based on the ability of antioxidants to reduce Fe(III) ions to Fe(II) ions in buffered sour medium (pH 3,6). The produced Fe (II) can be measured on photometers. Absorbance is proportional to the quantity of the produced Fe(II) ions and the antioxidants, respectively.

Our measurements showed more than threefold differences in total antioxidant activity among varieties, the lowest value being 171.13 μM/l and highest 702.57 μM/l. The corresponding betanin (17.18 and 57.80 mg/100 ml) and total polyphenol (37.5 and 85.5 mg/100 ml respectively) contents show similar differences. The highest FRAP values was measured in the *Bonel, Pablo* and *Pronto* varieties (506.97; 571.43; 702.57 μM/l). Based on our results it can be stated that varieties of higher betanin and poliphenol contents have higher antioxidant values as well. With the further measurements we concluded that red cabbage varieties greatly vary in pigment. There is a correlation between the pigment and dry matter content and FRAP. According to our data the highest FRAP parameters were measured in *Sandoro* F_1 whose colour intensity also proved to be excellent. Lower parameters were shown by *Rendero* F_1 which also lagged behind regarding dry matter content and pigment content.

Our measurements showed the varieties with higher pigment and poliphenol content have high antioxidant values too. There is a close correlation between red pigments (betanin), total polyphenol contents and FRAP values. The correlation between the quantity of these compounds and the FRAP values (r = 0.7799 and r = 0.7435, respectively).

Accordingly, the two compounds must have a role in the evolution of antioxidant effects.

Biological evaluation of volatile oils and aromatic agents by FRAP method

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Biological (antioxidant) values of volatile oils used in medication and basic components used in flavouring and parfumery have not been known so far. For that reason the authors examined the applicability of a chemical method (FRAP) adapted for plant materials. The aim of the work was to study that the volatile oils and essence of parfume of positive effect to biochemical (allergic) processes in the human body may posses any antioxidant properties.

Volatile oils, fragrance compositions (know and unknown combinations, but known tendency) were studied. The measurements were made in 1% solutions. The components of volatile oils and fragrance compositions were identified by GC-MS and FRAP method was used for measurement of antioxidant property.

The identified and frequently occuring basic-, aromatic- and perfumed compounds may be characterized by the following FRAP values: methyl salicylate 303±1 μmol/L, α-amyl acetate 144±2 μmol/L, borneol 284±1 μmol/L, camphor 286±1 μmol/L, carvon 164±2 μmol/L, menthol 1.86±0.23 μmol/L, menthon 1.88±1.10 μmol/L, thymol 284±1 μmol/L, linalol 299±1 μmol/L, linalyl acetate 221±4 μmol/L, limonene 445±3 μmol/L, terpineol 142±1 μmol/L, cinnamic aldehyde 303±1 μmol/L, anethol 509±2 μmol/L, while the FRAP values of volatile oils were the followings: lemon oil 198±1 μmol/L, geranium oil 152±2 μmol/l, sage oil 313±3 μmol/L, pine oil 255±1 μmol/L, muscate sage oil 484±2 μmol/L, patchuli oil 178±2 μmol/L, petitgrain oil 174±3 μmol/L, dill seed oil 323±1 μmol/L, eukalyptus oil 14±2 μmol/L, clove oil 197±1 μmol/L, peppermint oil 167±1μmol/L, rosemary oil 35 ±1 μmol/L. The FRAP values of essence-compositions varied between 250-1000 μmol/L (female 251±8 μmol/L, male 409±13 μmol/L, kid 1093±7 μmol/L). At the T1-T15 style tendency with unknown compositions the values were between 3000-9000 μmol/L in 10% solutions, while smaller values characterize the american unisex essences (P-18, P-19, P-20): 243±2 μmol/L, 252±4 μmol/L and 520±1 μmol/L, respectively.

It has been stated that the FRAP method is suitable for measurement of in vitro antioxidant property of complex compositions.

The FRAP values of volatile oils of the knowledge of main componenst help to estimate the quality of volatile oil with 20-30% deviation. In the case of fragrance compositions with known essence style tendency, the values show the reducing power and they do not contain any other information.

It is recommended to determine the antioxidant values of all aromatic agents, volatile oils and essence-compositions beside physico-chemical characteristics.

Modification of fully activated NADPH oxidase activity by antioxidants

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The first reactive oxygen-derived substance is the superoxide anion produced by NADPH oxidase. This is a multicomponents enzyme containing several cytosolic (phox proteins) and membrane-bound (Cytochrome b558) parts. NADPH oxidase can be activated by receptor-mediated and non-receptor-mediated ways. During the activation the cytosolic components are phosphorylated and translocated to membrane, where they are combined with the membrane-bound part of Cytochome b558. Cytochrome b558 consists of two parts, gp91phox and p22phox proteins. During the last few years it became clear that this complex is not able to produce superoxide anion, it requires coupling of a Rac 1 or 2 GTPase G protein to complex. When coupling of Rac 1/2 is inhibited the NADPH oxidase enzyme could not produce superoxide anion. Now, it is well demonstrated that NADPH oxidase can be found not only in phagocytes such as neutrophils, monocytes, but many other cells as well, they are called to NOX enzymes. NOX was identified in uterus, renal cells, hepatocytes, endothelial cells, lymphocytes, smooth muscle cells etc. Those non-phagocyte NADPH oxidases (NOX1, NOX3, NOX4) differ from phagocyte one (NOX2) in the structure of gp91 phox subunit. This difference results in that non-phagocyte NADPH oxidase produces smaller amounts of superoxide anion and its activation does not require activation of PKC.

During the last years, our group studied the effects of several natural and synthetic antioxidants on superoxide anion production and activation of PKC in human neutrophils. These examinations involved the study the effects of antioxidants on superoxide anion production by fully activated NADPH oxidase (NOX2) as well. During experiments we have found that some antioxidants can decrease it, while others have not such effects. It was also demonstrated that the effects of antioxidants on fully activated NADPH oxidase was independent on PKC inhibiton; and as a consequence, independent from the modification of superoxide anion production in intact neutrophils induced by antioxidants. These differences were the most pronounced in case of tocopherols and their water soluble metabolites (CEHC). Both tocopherols and CEHC-compounds inhibited PKC and superoxide anion production in phorbol-ester stimulated neutrophils, and CEHC were more effective inhibitors as parent tocopherols. In contrast, superoxide anion production by fully activated NADPH oxidase was only decreased by lipid-soluble tocopherols.

On the basis of our observations, we suggest the following mechanism for the action of antioxidants on superoxide anion production by fully activated NADPH oxidase: the bound between the enzyme complex and the small Rac 1/2 protein - which is necessary for superoxide anion production by NOX – might be slack or broken by antioxidants, which is due to the counteraction of lipid-soluble antioxidants and cell membrane. This observation might be useful in those clinical states when activation of NADPH oxidase occurs - in either phagocyte or non-phagocyte cells-, since in these cases we can choose antioxidants which are able to decrease superoxide anion production by fully activated NADPH oxidase(s) (NOX) and prevents development of oxidative stress.

Reaseach was sponsored by Fund of Hungarian Academy of Sciences (T 42550).

Volume 53(Suppl.1) 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Biological evaluation of alcoholic extracts of medicinal plants by the application of FRAP method

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Alcohol is often applied as a polar solvent for extraction of bioactive agents of medicinal plants. There are hardly any data on biological values, antioxidant properties of alcoholic extracts, therefore the aim of the work was to determine the antioxidant property/reducing ability of these kind of extracts. For the uniform dosage of agents, they could be hold on as a stock of family doctors, since the alcoholic extracts of herbs choosen are official in the Hungarian Pharmacopoeia (Ph.Hg.VII). Alternative medication, e.g. homeopathy, also often use tictures of alkaloid containing, when the diluted alcoholic solution is applied for symptomatic treatment. The data according to the examination may help to estimate the dose used and the effect in both modern and alternative medication point of view.

The following alcoholic extracts were prepared by the description of Hungarian Pharmacopoeia: Tinctura Amara, Tinctura Chamomillae, Tinctura Chinae, Tinctura Ipecacuanhae, Tinctura Ratanhiae, Tinctura Saponariae, Tinctura Strychni, Tinctura Thymi, Tinct Stramonii. For examination, the extracts of 40% concentration were used.

The measurement of biological active components in extracts was performed according to the Hungarian Pharmacopoeia (Ph. Hg VII) and the determination of antioxidant values was realized by FRAP method. The method is based on the transformation of Fe^{3+} into Fe^{2+} at low pH value. Formation of Fe^{2+}-TPTZ (2,4,6-tripyridyl-S-triazine) complex is traceable by spectrometry based on color reaction at 593 nm.

The quality and quantity characteristics of extracts are agreed with the description of Ph.Hg. VII. The FRAP values of alcoholic extracts are the followings: Tinctura Amara 768.5 µmol/L, Tinctura Chamomillae 741.9 µmol/L, Tinctura Chinae 642.9 µmol/L, Tinctura Ipecacuanhae 281.8 µmol/L, Tinctura Ratanhiae 427.0 µmol/L, Tinctura Saponariae 686.1 µmol/L, Tinctura Strychni 482.9 µmol/L, Tinctura. Thymi 177.7 µmol/L, Tinctura Stramonii 832.3 µmol/L.

It is allocated that this method can be apply as the expression of vegetable alcohol-water extract of biological values.

It has been stated that the FRAP method is suitable for evaluation of biological values of alcoholic-aqueous extracts.

Author Index

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Coons AH (1978) Fluorescent antibody methods. In Danielli JF, ed., General Cytochemical Methods. Academic Press, New York, 399-422.

Maxam AM, Gilbert WA (1977) A new method for sequencing DNA. Proc Natl Acad Sci USA 74:560-564.

Monod J, Changeux J-P, Jacob F (1963) Allosteric proteins and cellular control systems. J Mol Biol 6:306-329.

Schwarz-Sommer Z, Huijser P, Nacken W, Saedler H, Sommer H (1990) Genetic control of flower development by homeotic genes in Antirrhinum majus. Science 250:931-936.

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